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IX. FLUORIDE

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This document is a review of the scientific literature on the biological and environmental effects of fluoride. Included in the review are a general summary and a comprehensive discussion of the following topics as related to fluoride and specific fluorine-containing compounds: physical and chemical properties; occurrence; synthesis and use; analytical methodology; biological aspects in microorganisms, plants, wild and domestic animals, and humans; and distribution. mobility, and persistence in the environment. The document also contains an evaluation of potential hazards resulting from fluoride contamination of the environment and suggests current research needs. More than 1000 references are cited.

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FOREWORD

A vast amount of published material is accumulating as numerous research investigations are conducted to develop a data base on the adverse effects of environmental pollution. As this information is amassed, it becomes continually more critical to focus on pertinent, well-designed studies. Research data must be summarized and interpreted in order to adequately evaluate the potential hazards of these substances to ecosystems and ultimately to public health. The Reviews of the Environmental Effects of Pollutants (REEPs) series represents an extensive compilation of relevant research and forms an up-to-date compendium of the environmental effect data on selected pollutants.

Reviews of the Environmental Effects of Pollutants: IX. Fluoride includes information on chemical and physical properties; pertinent analytical techniques; transport processes to the environment and subsequent distribution and deposition; impact on microorganisms, plants, and wildlife; toxicologic data in experimental animals including metabolism, toxicity, mutagenicity, teratogenicity, and carcinogenicity; and an assessment of its health effects in man. The large volume of factual information presented in this document is summarized and interpreted in the final chapter, "Environmental Assessment," which presents an overall evaluation of the potential hazard resulting from present concentrations of fluoride in the environment. This final chapter represents a major contribution by James L. Shupe, A. E. Olson, and H. B. Peterson of Utah State University.

The REEPs are intended to serve various technical and administrative personnel within the Agency in the decision-making processes, i.e., in the development of criteria documents and environmental standards, and for other regulatory actions. The breadth of these documents makes them a useful resource for public health personnel, environmental specialists, and control officers. Upon request these documents will be made available to any interested individuals or firms, both in and out of the government. Depending on the supply, the document can be obtained directly by writing to:

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ABSTRACT

This study is a comprehensive, multidisciplinary review of the health and environmental effects of fluoride on microorganisms, plants, wild and domestic animals, and humans. More than 1000 references are cited.

Fluoride is widely distributed in the environment, occurring in igneous rocks (210 to 1000 ppm), sedimentary rocks (180 to 940 ppm), normal soils (200 to 300 ppm), surface waters (uncontaminated lakes and streams, <0.3 ppm), seawater (approximately 1.5 ppm), and air (nonindustrial areas, <0.05 μg/m³). Most fluoride emissions to the atmosphere occur during the manufacture of phosphorus and phosphate fertilizer, the operation of aluminum- and steel-producing furnaces, the production of brick and tile products, and the combustion of coal. Liquid-fluoride wastes are generated primarily during the production of glass products, pesticides, fertilizers, aluminum, steel and inorganic chemicals, and in metal-processing industries.

Human intake of fluoride is chiefly through the diet; drinking water is normally the largest single source. Low concentrations of fluoride in water (approximately 1 ppm) benefit mammalian systems, making bone and tooth apatite less soluble, but long-term ingestion of water containing more than 8 ppm fluoride causes fluorosis in humans. Fluoride salts are lethal to humans when ingested in doses of about 3 g or more. At concentrations normally encountered by the general public, fluoride is not teratogenic, mutagenic, or carcinogenic to humans, but chronic fluoride toxicosis of both livestock and wildlife is an important problem in many areas of the United States. The principal manifestations of chronic fluoride toxicosis in livestock are dental fluorosis, osteofluorosis, lameness, and impaired performance. Among domestic animals, dairy cattle are the most sensitive to excessive fluoride exposure.

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SECTION 1

SUMMARY

1.1 DISCUSSION OF FINDINGS

1.1.1 Chemical and Physical Properties and Analytical Techniques

Fluorine is a pale yellow acrid gas that freezes to a colorless solid at -219.6°C and boils at -188.2°C; it is a common element, ranking 13th in abundance and constituting 0.06% to 0.09% of the earth's crust. Fluorine is highly reactive and rarely exists in the elementary state in nature. It usually occurs as ionic or covalently bonded fluoride. The most common chemical forms are hydrogen fluoride, alkali fluorides, silicon tetrafluoride, sodium fluorosilicate, fluorocarbons, uranium hexafluoride, chalcogen and halogen fluorides, and the minerals cryolite, fluorapatite, and fluorspar (Section 2.2).

Techniques for sampling environmental sources for fluoride are well established, except for the partitioning of airborne gaseous and particulate fluorides. A variety of good analytical methods are available for determining fluoride in environmental media. The widely used spectrophotometric procedure based on the zirconyl-SPADNS reagent is now being rapidly superseded by the relatively new fluoride ion electrode. The latter technique has comparable, or better, sensitivity (1 to 100 ng/g), precision (1% to 10%), and accuracy (1% to 5%) and is quicker and more convenient than other methods for most types of fluoride samples. Fluoride in environmental samples can also be determined by polarographic, enzymatic, and activation analyses techniques, but these methods are competitive with the fluoride ion electrode method only under special circumstances (Section 2.3.3).

The biochemistry of fluorides is complex. Drinking water concentrations of about 1 ppm fluoride are beneficial to mammals, reducing the dissolution of hydroxyapatite crystals in teeth and bone through the formation of the less soluble fluorapatite. About 99% of the fluoride retained in the human body is localized in this fashion. However, higher levels of fluoride are toxic to all biological systems. The manner in which excess fluoride interferes with biochemical processes is not well understood at the molecular level. It is known, nevertheless, that excess fluoride prevents oxidative metabolism by inhibiting the action of enzymes that depend on polyvalent cations such as magnesium, calcium, iron, and manganese. In addition, other body functions that require complexable polyvalent metal ions (e.g., membrane transport, nerve conduction, muscle contraction, and blood clotting) are also disrupted. It is apparent that the fluoride ion, per se, is responsible for the toxic effects. Accordingly, soluble inorganic fluoride salts are more toxic than insoluble salts, and most organic compounds that do not yield free fluoride ions in body fluids have little or no toxicity. The naturally occurring organic compounds fluoroacetic acid and ω-fluorooleic acid are exceptions to this rule; their highly toxic effect is due not to free fluoride ion but to their metabolic conversion to fluorocitric acid, which interferes with the chemistry of the Krebs cycle, causing metabolic death of the cell. Treatment of acute fluoride intoxication is based on reducing the cellular availability of fluoride through the formation of complexes or precipitates in the intestinal tract or in the bloodstream.

1.1.2 Environmental Occurrence

Fluoride is widely distributed in the environment. It occurs most abundantly in ores, such as fluorspar (CaF_2); in phosphate and silicate minerals, such as fluorapatite [$CaF_2 \cdot 3Ca_3(PO_4)_2$]; and in topaz [$Al_2SiO_4(F,OH)_2$]. It is also present in lesser amounts in most igneous (210 to 1000 ppm) and sedimentary (180 to 940 ppm) rocks. Normal mineral soils average 200 to 300 ppm fluoride. Generally, sandy soils contain less than average amounts of fluoride while heavier soils contain more. Soils naturally rich in phosphorus tend to contain above-average fluoride concentrations — in extreme cases, levels of 7000 to 8000 ppm are observed (Section 7.3.2.2).

Fluoride is a normal constituent of natural waters. The fluoride content of surface water depends on the water source and the amount of precipitation received; normally, fluoride in uncontaminated lakes and streams does not exceed 0.3 ppm. Seawater usually contains more fluoride than fresh surface water — about 1.4 to 1.5 ppm in waters of normal salinity. Highly saline waters have increased concentrations of fluoride; the Great Salt Lake in Utah and certain lakes in Kenya, Africa, contain 14 and 1600 ppm fluoride respectively. Fluoride in groundwaters, such as springs, wells, and infiltration galleries, varies greatly depending on the type of rock the water flows through. Groundwaters associated with alkalic igneous rock, dolomite, phosphorite, and volcanic glasses contain fluoride concentrations that usually do not exceed 10 ppm but may occasionally reach 60 to 70 ppm. Large areas of the world have local groundwaters with more than 1.5 ppm fluoride. Limits set by the U.S. Public Health Service for fluoride in drinking water vary from 0.6 to 1.7 mg/liter, depending on local air temperatures. Nearly all U.S. public water supply systems meet this standard (Section 7.3.3).

Fluorides occur naturally in the atmosphere; soluble gaseous fluorides and soluble and insoluble dusts are formed as a result of the weathering of rocks and minerals, volcanic activities, and precipitation. The concentrations of these materials in ambient air depend on the amount of fluoride emitted, the distance from the source, meteorological conditions, and the topography of the area. In both rural and urban areas, atmospheric fluoride concentrations are typically reported as less than 0.05 $\mu g/m^3$, the limit of detection of the analytical method used in the survey. However, higher localized atmospheric concentrations of fluoride frequently result from industrial activities, particularly the combustion of coal, the manufacturing of aluminum and steel, and the production of phosphate fertilizer. U.S. coals contain about 60 ppm fluoride. Half of this fluoride escapes to the atmosphere during combustion as hydrogen fluoride, silicon tetrafluoride, or fluoride particulates. Similar contaminants are released during phosphate fertilizer production, and average fluoride concentrations in excess of 3 $\mu g/m^3$ have been reported in the air near older phosphate fertilizer manufacturing plants. These concentrations are well below the threshold limit values of 2.5 and 0.2 mg/m^3 set for fluoride and fluorine in the workplace, respectively, by the American Conference of Governmental Industrial Hygienists (Section 7.3.4).

Hydrogen fluoride is the most important manufactured fluoride; it is the intermediate from which all other fluorides are prepared. In 1974, the U.S. consumption of all forms of fluorides amounted to 625,100 metric tons (689,000 short tons), computed as fluorine. About one-third of this total (32.5%) was consumed in synthesizing organic compounds and products such as dichlorodifluoromethane, trichlorofluoromethane, tetrafluoromethane, tetrafluoroethylene, vinyl fluoride, and hexafluoropropene, which were used for aerosol propellants, refrigerants, and fluorinated plastics. The remaining fluoride was used primarily by metal producing or processing industries; steel and electrometallurgical fluxing, 44%; nonferrous metal production, 22%; ceramics and glass manufacture, 1.3%; and other processes, 0.2% (Section 7.2).

Large quantities of chlorine-containing fluorocarbons released to the atmosphere in recent years as aerosol propellants or refrigerants raised questions about destruction of stratospheric ozone and ensuing health hazards from increased ultraviolet radiation. The extent to which this reaction occurs is uncertain, but studies to establish the significance of the reaction are in progress. As a precautionary measure all nonessential aerosol propellant uses of fluorocarbons were banned by federal regulations in 1978.

Consumption of fluorine in the United States is increasing rapidly and is expected to more than triple by the year 2000 (Section 7.2).

1.1.3 Environmental Cycling and Fate

Fluoride cycles naturally through the environment. It moves from the lithosphere to the atmosphere primarily by volcanism and entrainment of soil particles (Section 8.2), but manufacturing activities are becoming increasingly significant. Volcanism is estimated to transfer 10^9 kg of fluoride per year to the atmosphere. In 1970, emissions from U.S. industrial activities amounted to an additional 1.5×10^8 kg of fluoride.

Atmospheric fluoride returns to the hydrosphere and lithosphere in precipitation and by deposition of particulates. Rainfall, which averages 0 to 0.02 ppm fluoride in uncontaminated areas, contains 0.2 to 14 ppm fluoride in areas near urban and industrial sources. In one location where much coal is burned, rainwater is estimated to add 170 g of fluoride per hectare annually. Global precipitation is estimated to deposit 1.2×10^{10} kg of fluoride per year (Section 7.3.1.2.2).

Fluoride tends to persist in most soils. It is strongly absorbed by soil colloids and is not easily displaced by common anions. In alkaline soils fluoride is usually fixed as the insoluble calcium salt, or, if adequate calcium is not present, as aluminum silicofluoride. In very alkaline soils the sodium salt may be formed. Losses from runoff and leaching are relatively small averaging 0.5% to 6% annually in typical forest and agricultural areas (Section 7.4.1).

Dissolved and particulate fluorides from natural and industrial sources are transported to the sea primarily by flowing water; as a consequence, world rivers average about 0.09 to 0.2 ppm fluoride (Section 7.3.3). Seawater contains ten times as much fluoride as rivers. Oceanic sediments are richer still, averaging about 730 ppm. Some 4 to 6 × 10^{11} g of fluoride is withdrawn from the sea annually by incorporation into calcium carbonate or calcium phosphate. This removal results in an average fluoride residence time in the ocean of 2 to 3 million years. There is no apparent difference in the fluoride content of sediments from the Atlantic, Pacific, and Indian oceans (Section 7.4.2).

Several effective and economical treatments exist for removing fluoride from gaseous- and liquid-waste streams. Fluorides are usually scrubbed from gaseous wastes with water or caustic solutions. The most commonly used methods for removing fluoride from liquid wastes involve precipitation of calcium or aluminum fluoride by addition of lime or alum, followed by filtration or settling (Section 7.5). Another frequently used waste management technique involves absorption of ionic fluoride on ion exchangers or alumina. The fluoride sludge resulting from these operations is normally disposed of as landfill. Adequate precautions against acidification of these landfill sites are necessary to avoid inadvertent release of solubilized or vaporized fluorides.

1.1.4 Biological Aspects in Microorganisms

Many microorganisms, such as bacteria, fungi, yeast, algae, protozoa, and viruses, are known to metabolize fluoride or fluoride-containing compounds. A few strains of bacteria can utilize fluoride compounds as catabolites. Microorganisms may contain relatively high concentrations of fluoride. Some samples of oral bacteria contain up to 134 ppm, and accumulations up to 900 ppm fluoride may occur in lichens grown near an industrial pollution source such as an aluminum smelter. In most instances, fluoride appears to be only loosely bound to microbe cells and can be removed or reduced in concentration by washing with water or other solvents (Section 3.2). There is no evidence that fluoride is essential to microorganisms. In fact, excessive concentrations are toxic, but different species exhibit different tolerances. Toxic effects of fluoride on microorganisms include developmental and morphological alterations, growth inhibition, and reduction in infectivity. These effects occur primarily through interference with respiratory, photosynthetic, ionic transport, and glycolysis processes. Higher molecular weight compounds and compounds containing more chlorine than fluorine seem to be most toxic to microorganisms. Dormant bacterial spores are more resistant than vegetative cells to fluoride aerosols (Section 3.3). The laboratory substitution of *p*-fluorophenylalanine and 5-fluorouracil for their unfluorinated analogs in bacterial proteins and nucleic acids interferes with subsequent vital biological processes, such as protein reduction, RNA and DNA synthesis, and cell differentiation; it also causes chromosomal alterations, changes in ribosomal composition and properties, and modification in functions of messenger RNA. There is no evidence, however, that mutagenic effects to microorganisms occur by chance, from simple fluoride compounds normally encountered in the environment (Section 3.3.1).

1.1.5 Biological Aspects in Plants

Fluoride is found in virtually all plants. In unpolluted areas, fluoride concentrations normally range from about 2 to 20 μg per gram of dried plant matter. Near industrial pollution sources, such as aluminum or phosphate fertilizer producers or in soils of high fluoride content, concentrations of fluorides in plants may be 10 to 100 times greater (Section 4.2.4.2). Although plants can absorb fluoride from soil, levels of major nutrients in the soil affect the amount of fluoride absorbed through roots, and there is not necessarily a simple direct relationship between concentrations of fluoride in soil and plant tissues. Uptake of fluoride by plants is strongly pH dependent (Section 4.2.1.1). Plants absorb gaseous and airborne particulate fluorides via leaves; in areas with polluted atmospheres most of the fluoride in the plant is probably supplied in this manner. Gaseous fluorides are more effectively absorbed than particulate forms and lead to greater plant injury. Tissue fluorides increase with increased length of exposure and with increased atmospheric concentrations. Existing data are inadequate to determine subcellular sites of localization; however, some evidence suggests that chloroplasts accumulate fluoride to a greater extent than mitochondria or cell walls. Mobility of fluoride within the plant is limited — fluoride is translocated upward from root to shoot, but leaf fluoride is essentially fixed. Bioelimination occurs through loss of leaves, twigs, and roots, leaching by rain, and in certain plants possibly by volatilization of organofluorides (Section 4.2.5).

The metabolism of organic fluoride by plants is not well understood. Some plant species synthesize and accumulate large amounts of compounds such as fluoroacetate. A variety of plants form trace amounts of fluoroacetate and fluorocitrate, but no information is available concerning enzyme systems necessary to synthesize or degrade these organofluorides. It is also uncertain if all plants can synthesize carbon-fluorine bonds (Section 4.2.3).

Although the linear growth of selected plants may be enhanced by exposure to a few parts per billion of airborne fluoride, the presence of fluoride in plant tissue in abnormal concentrations is generally detrimental. All plants are not equally susceptible; gladiolus, apricot, and Douglas fir are exceptionally sensitive while cherry, tomato, and wheat are resistant. Excess fluoride causes growth inhibition, tip and marginal necrosis of foliage, chlorosis, wilting, and eventually death of the plant. Fruit quality and yield can be impaired. Inhibition of seed germination also occurs. Fluoride causes these effects by altering photosynthesis, carbohydrate metabolism, respiratory and oxidative processes, RNA metabolism, and calcium nutrition. The mechanisms involved are complex and largely unresolved in vivo, but in vitro studies suggest that many effects result from fluoride inhibition of essential enzymatic reactions or effects on the structural integrity of the cell (Section 4.3.1).

Fluoride appears to be mutagenic in plants. Chromosomal abnormalities, such as breakage, bridging, and stickiness, occur in a variety of plants after treatment with concentrations of gaseous hydrogen fluoride or aqueous sodium fluoride too low to induce immediate visible injury. The mechanisms by which fluoride induces chromosomal aberrations are not known (Section 4.3.4).

1.1.6 Biological Aspects in Animals

Animals are exposed to a variety of forms and concentrations of fluoride through ingestion of food and water and from the atmosphere. Ingestion is the principal form of uptake by insects, aquatic and terrestrial animals, and birds; inhalation and skin absorption contribute only negligible amounts. Fluorides are metabolized and accumulate in mineralizing tissues of animals. Usually, 96% to 98% of the fluoride in the body is located in bones and teeth. Although most mammals normally accumulate fluoride in mineralizing tissues with age, the major portion of that assimilated is rapidly excreted in urine. Smaller amounts are eliminated in feces, saliva, and perspiration (Section 5.5.1.3).

Tolerance to fluoride differs among various animal species and among individuals of the same species. Birds tolerate fluorides better than mammals (Section 5.4.2.2.4). Based on performance, growing chicks accept up to 300 ppm fluoride in their diet without difficulty; turkeys up to 400 ppm. However, teratogenic effects occur when fluoride-containing compounds (e.g., 5-fluorouracil, 5-fluoro-2'-deoxyuridine, and 5-fluoroorotic acid) are injected into the yolk sac during the first four days of development of chicken embryos. Skeletal deformation, microphthalmia, retardation of growth of extremities, and abnormal bill development are common malformations caused by intrayolk injections (Section 5.4.2).

Fluoride toxicosis of livestock usually occurs only in areas with a fluoride pollution problem, but significant exposures can result from the ingestion of excessive raw rock phosphate and geothermal waters rich in fluorides (Section 5.5.1). Acute fluoride toxicosis is rare, but chronic fluoride toxicosis occurs in both livestock and wildlife and is an important problem in the United States. Dental and bone lesions, lameness, and impaired performance are the principal manifestations of chronic fluoride toxicosis in livestock. Sheep and horses are more tolerant to fluoride than cattle. Based on chemical signs and lesions, safe levels of fluoride in the feed of these animals are 60 ppm to 30 ppm, respectively, under breeding or lactating conditions. Swine appear to be the most tolerant among domesticated mammals, tolerating up to 70 ppm fluoride in their feed (dry basis) without apparent injury (Section 5.5.2.4). Feed values should be reduced proportionally when water contains appreciable amounts of fluoride. Available scientific data do not support carcinogenic effects of fluorides in wild or domestic animals. Water characteristics, such as pH, hardness, and contaminants, influence the tolerance of aquatic animals to fluoride; sensitivity is also related to environmental acclimatization and species of animal. In general, however, concentrations of 1.5 ppm fluoride appear not to have harmful effects, although slightly higher levels may be detrimental to some aquatic species. For example, the 20-day median tolerance limit for some Salmo fingerlings is 2.7 to 4.7 ppm sodium fluoride at 12.8°C, under constant-flow conditions. Filter-feeding molluscs are also very sensitive to fluoride. Mussels subjected to seawater containing 10 ppm fluoride die after five-week exposures. Growth of crustaceans (e.g., the crab) is reduced by exposure to water containing 20 ppm fluoride. Muscle tissue of such animals can accumulate up to 50 ppm fluoride — a level potentially hazardous for continual human use. Since fluoride concentrations of 20 ppm have been reported in estuaries where phosphate mining operations occur, such operations can have adverse effects on the crabbing industry. It is worth noting, however, that the accumulation of fluoride in crustaceans is reversible: both hard and soft tissue concentrations approach near-normal levels following exposure of these animals to fluoride-free water for a time comparable with the contamination period (Section 5.3.2.1).

Both inorganic and organic fluorides produce lethal and sublethal toxicity in insects. The LD_{50} for many different species varies from 100 to 300 mg of sodium fluoride per kilogram of living weight. Exposure to fluoride causes either a stimulation or an inhibition of egg production in insects, depending on the length of exposure and concentration of the poison. For example, seven-day exposure of some *Tribolium* larvae to flour containing 0.01% sodium fluoride stimulated egg production, compared with controls, but a similar exposure to flour containing 0.1% sodium fluoride inhibited egg production. Sublethal exposures of fluoride may also cause mutagenic effects in some insects; thus increased sex-linked recessive lethal mutations occur in *Drosophila melanogaster* males after exposure to a mixture of gas containing 10% perfluorobutene-2 and 90% air (Section 5.2.2.1.3).

1.1.7 Biological Aspects in Humans

The biological response of humans to fluoride depends on many factors, such as the chemical form and concentrations involved. At trace levels ionic fluoride may be essential; at intermediate concentrations (approximately 1 ppm) it appears beneficial; and at higher levels it is toxic. The essentiality of fluoride is difficult to show experimentally because of its ubiquity and the resulting difficulty in removing fluoride from the diet; nonetheless, trace levels of this substance may be necessary for nucleation of crystalline material in bone (Section 6.2). Although fluoridation of public water supplies has caused controversy, most evidence indicates that this step is beneficial and is the most cost-effective and safest procedure of improving dental health (Section 6.4.2). An even higher level of fluoride than the 1.0 ppm considered optimum for caries resistance may enable older persons to better resist osteoporosis (Section 6.4.2.3).

Human intake of fluoride is chiefly through the diet, drinking water usually furnishing the largest share. Absorption of fluoride is largely passive; the amounts retained depend on the solubility of the fluoride compound (Section 6.3.1.2). Absorbed fluoride is rapidly distributed across membranes and into tissues; however, bone is the major storage site. Bones accumulate fluoride as fluorapatite or possibly, to a lesser extent, as magnesium fluoride, throughout the lifetime of an individual. The rate decreases with age (Section 6.3.2.2). Fluoride sequestered by bone during periods of excess intake is released to the blood and excreted through the kidneys when the level of intake subsides. Some fluoride is eliminated in sweat, and small amounts pass through the mucosa of the intestines into the gut.

Long-term ingestion of water containing fluoride at levels above 8 ppm causes skeletal fluorosis osteofluorosis, in about one in ten persons. In most cases, the skeleton does not deteriorate and functions normally, both structurally and metabolically. When fluorosis is severe, bone changes occur and may be accompanied by crippling, impaired locomotion, and pain. Fluoride salts are lethal when taken orally in doses of about 3 g (Section 6.4.3). Aspects of fluoride poisoning leading to death are blockage of respiratory enzymes, interference with necessary body functions controlled by calcium, specific organ damage, and general collapse (Section 6.4.3.2). Some of these effects are reversible. If proper prompt treatment is provided, not only may the patient be saved, but recovery without sequelae is possible. In the case of exposure to HF or F_2, the extremely corrosive nature of these substances adds further damaging effects to their general fluoride toxicity (Section 6.4.3.3). Absorbed as fumes or vapors, they cause extreme irritation to the lungs, resulting in edema and tissue damage as well as systemic toxic effects.

Except for accidental ingestion of fluoride salts, most U.S. residents are exposed to fluorides chiefly from industrial or occupational contacts. Usually, threshold limit values have been established for such exposures, and exposure levels are monitored by air, food, and water sampling, and urine analyses. At concentrations normally encountered by the general public, fluoride is not teratogenic, mutagenic, or carcinogenic (Section 6.4.4). However, some concern exists over possible teratogenic or other reproductive effects of fluorinated anesthetics among chronically exposed persons such as operating room personnel (Section 6.4.3.6.2). Continued study of the risk-benefit factors for such uses appears warranted.

1.1.8 Food Web Interactions

The movement of natural fluorides in the food chain has been examined only in a very preliminary manner and is not well established. The available data indicate a 20- to 50-fold increase of fluoride in the femurs of herbivores (e.g., deer mice) relative to forage values, and smaller increases — perhaps 2- to 5-fold — in skeletons of predators of these herbivores. The increased uptake of fluoride in the diets of predators presumably results from consumption of prey skeletons since only trace levels of fluoride occur in the soft tissues. Because the fractional consumption of skeletal tissues by predators decreases higher in the food chain, localization of fluoride in skeletal tissues effectively limits the transfer of fluoride in most species, and biomagnification is not a serious problem. Thus animals grown for human consumption generally do not significantly increase human uptake of fluoride.

1.2 CONCLUSIONS

1. Fluoride occurs in detectable concentrations in most rocks, soils, and waters, as well as plant and animal tissues.
2. Normal mineral soils contain 200 to 300 ppm fluoride.
3. Uncontaminated surface waters usually contain no more than 0.3 ppm, but seawater of normal salinity averages 1.4 to 1.5 ppm fluoride.
4. Most urban and almost all nonurban air contains environmentally insignificant amounts of fluoride ($<0.05\ \mu g/m^3$).
5. Fluorite (CaF_2) is the most abundant and economically important fluoride mineral.
6. Hydrogen fluoride (HF) is the most important manufactured fluoride and is probably the greatest single industrial fluoride contaminant of the atmosphere.
7. Most fluoride emissions to the atmosphere occur in the manufacturing of phosphorus and phosphate fertilizer, the operation of aluminum- and steel-producing furnaces, the production of brick and tile products, and the combustion of coal.
8. Liquid-fluoride wastes are generated primarily in the production of glass products, pesticides, fertilizers, aluminum, steel and inorganic chemicals, and in metal-processing industries.
9. Fluoride is usually removed from aqueous wastes by the addition of lime or alum, followed by filtration of the resulting calcium or aluminum precipitate, or by absorption on ion exchangers or alumina.
10. Volatile fluorides are usually fixed by scrubbing gaseous wastes with water or caustic solutions.
11. Good analytical methods are available for determining fluoride in environmental samples at concentrations down to the parts-per-billion level.
12. Low concentrations of fluoride in water (approximately 1 ppm) benefit mammalian systems, making bone and tooth apatite less soluble.
13. Excess fluoride reacts with some complexable polyvalent cations in biological systems, preventing normal enzymatic reactions and causing cellular death.
14. Ingestion is the principal form of uptake of fluoride by insects, aquatic and terrestrial animals, and birds; inhalation and skin absorption contribute only negligible amounts.

15. Acute fluoride toxicosis of livestock is rare, but chronic fluoride toxicosis of both livestock and wildlife is an important problem in many areas of the United States.

16. The principal manifestations of chronic fluoride toxicosis in livestock are dental fluorosis, osteofluorosis, lameness, and impaired performance among domestic animals. Dairy cattle are the most sensitive to excessive fluorides.

17. Fluoride accumulates in the hard tissues of animals — approximately 98% of the body burden is incorporated in teeth and bones.

18. Concentrations of 1.5 ppm fluoride in seawater appear not to have harmful effects on marine animals, but higher levels are frequently detrimental.

19. Plants absorb some soluble fluoride from the soil via roots, while atmospheric gases and particulates are absorbed in and collected on leaves and stems. The latter mechanism predominates in areas near aluminum, steel, and fertilizer manufacturers.

20. Excess fluoride in or on plants causes growth inhibition, plant damage, reduced quality and yield of fruit, and fluoride toxicosis in grazing animals.

21. The metabolism of fluoride by plants is not well understood; however, it is established that excess fluoride in plants interferes with photosynthesis, carbohydrate metabolism, respiratory and oxidative processes, RNA metabolism, and calcium nutrition.

22. There is not necessarily a simple, direct correlation of fluoride in soil and plant tissues. Uptake is strongly pH dependent.

23. The LD_{50} for many species of insects ranges between 100 and 300 mg of sodium fluoride per kilogram of living weight.

24. Biomagnification of fluoride occurs at the lower end of the food chain but is limited by localization of fluoride in skeletal tissues. Therefore, it does not significantly increase human uptake.

25. Human intake of fluoride is chiefly through the diet (drinking water).

26. Water containing approximately 1 ppm of fluoride appears beneficial to human life, but higher concentrations may be toxic.

27. Long-term ingestion of water containing more than 8 ppm of fluoride causes fluorosis in humans.

28. Fluoride salts are lethal to humans when ingested in doses of about 3 g.

29. Except for accidental ingestion of salts, most U.S. residents are exposed to fluorides chiefly from industrial or occupational contacts. However, waters with high fluoride content are important sources of exposure in some countries such as India and Africa.

30. At concentrations normally encountered by the general public, fluoride is not teratogenic, mutagenic, or carcinogenic to humans.

31. Continued study of the risk-benefit factors for the use of fluorinated anesthetics is warranted.

SECTION 2

CHEMICAL AND PHYSICAL PROPERTIES AND ANALYSIS

2.1 SUMMARY

Fluorine is a common element, ranking 13th in abundance and constituting 0.06% to 0.09% of the earth's crust. Because of its small atomic radius and large number of electrons, fluorine has the greatest surface charge density of any element; consequently, it is highly reactive and only rarely occurs naturally in elemental form. Fluorine is widely disseminated, however, in ionic or combined forms; it appears in detectable concentrations in most rocks, soils, waters, and plant and animal tissues. Even rural air samples normally carry traces of fluoride.

Fluorine and its derivatives have large and growing uses in many industrial processes and products, and various fluorides occur as byproducts or wastes. The chemical forms most frequently encountered are fluorine gas, hydrogen fluoride, alkali fluorides, silicon tetrafluoride, sodium fluorosilicate, fluorocarbons, uranium hexafluoride, Group VIA element fluorides, halogen fluorides, and the minerals cryolite, fluorapatite, and fluorspar.

On the basis of quantity produced, hydrogen fluoride is the most important manufactured fluoride (292,000 metric tons in 1977); it is the intermediate from which all other fluoride compounds, including fluorine, are prepared. Hydrogen fluoride is obtained by treating the mineral fluorspar with sulfuric acid. Because of its extensive use, hydrogen fluoride is probably the greatest primary fluoride contaminant of the atmosphere. Owing to its great reactivity, however, it is unlikely to remain in this form very long.

Fluorination of organic compounds and products constitutes the greatest single use of fluorides in the United States. In 1977, approximately 108,000 metric tons of hydrogen fluoride was used in the synthesis of dichlorodifluoromethane, trichlorofluoromethane, tetrafluoromethane, tetrafluoroethylene, vinyl fluoride, hexafluoropropene, and similar products. These compounds are used chiefly for aerosol propellants, refrigerants, and fluorinated plastics. In 1978, nonessential uses of fluorocarbon propellants were banned, and this segment of the fluorocarbon market shrank to about 2% of its previous value. Smaller quantities of other fluorocarbons find specialized uses as inhalation anesthetics, fire extinguishing agents, cleaners, and degreasers. Other large users of hydrogen fluoride include the aluminum industry, uranium isotope enrichment plants, the petroleum industry, and stainless steel pickling operations.

Most fluoride emissions to the atmosphere occur in the manufacturing of fertilizer and phosphorus from rock phosphate, the operation of aluminum- and steel-producing furnaces, the manufacturing of brick and tile products, and the combustion of coal. In general, these wastes take the form of hydrogen fluoride, fluorine, boron trifluoride, fluorosilicic acid, sodium fluorosilicate, aluminum fluoride, calcium fluoride, lead difluoride, fluorapatite, silicon tetrafluoride, and fluoride particulates. Liquid-waste streams containing appreciable quantities of fluoride are generated by glass manufacturers, pesticide and fertilizer producers, steel and aluminum makers, metal processing industries, and inorganic chemical producers. Usually this fluoride is in the form of hydrogen fluoride or fluoride ion, depending on the pH of the waste stream.

Techniques for sampling environmental sources for fluoride are reasonably well established, except for the partitioning of airborne gaseous and particulate fluorides. A variety of good analytical methods are available for determining fluoride in these samples. The widely used spectrophotometric procedures based on the zirconyl-SPADNS, or similar, reagent are now being rapidly superseded by the relatively new fluoride ion electrode. The latter technique has comparable or better precision and accuracy and is more rapid and convenient than previous analytical methods for most types of fluoride samples. Fluoride in environmental samples can also be determined by polarographic, enzymatic, and activation analysis techniques. However, these methods are competitive with the electrode technique only under specialized circumstances.

2.2 PHYSICAL AND CHEMICAL PROPERTIES

Fluorine is unique among the elements in the variety of forms its compounds can take; many inorganic gases and salts exist, as well as a large and growing number of organic compounds. Some of these compounds interact significantly with the environment (Cholak, 1959*a*, 1959*b*) and are pertinent to this study; the most important of these are fluorine, hydrogen fluoride, alkali fluorides, fluorspar, cryolite, fluorapatite, silicon tetrafluoride, sodium fluorosilicate, fluorocarbons, organic fluorides, and Group VIA fluorides. Pertinent physical and chemical properties of these compounds are discussed in the following sections.

2.2.1 Fluorine

Fluorine ranks 13th in abundance among the elements and constitutes 0.06% to 0.09% of the earth's crust (Leech, 1956, p. 3). First isolated in 1886 by Moissan, elemental fluorine remained largely a laboratory curiosity until World War II, when nuclear energy requirements stimulated commerical production (Weast, 1978). It is now produced in high tonnages.

2.2.1.1 Physical Properties — Fluorine (F_2) is a pale yellow acrid gas that freezes to a colorless solid at -219.6°C and boils at -188.2°C; its heat of formation and energy of dissociation are 18.9 and 37.7 kcal/mole respectively (Nikolaev et al., 1972). The density of the gas is 1.69 g/liter (15°C, 1 atm); at -188°C, the density of the liquid is 1.51 (Weast, 1978), and the dielectric constant is 1.517 at -189.95°C (Neumark and Siegmund, 1966). Physical properties of fluorine are listed in Table 2.1.

TABLE 2.1. PHYSICAL PROPERTIES OF FLUORINE

Property	Value
Melting point (mp)	-219.62°C
Boiling point (bp)	-188.14°C
Transition temperature, solid	-227.61°C
Critical temperature	-129.00°C
Critical pressure	55 atm
Density, solid	1.90 g/cm^3
Refractive index, liquid at bp	1.2
Surface tension, liquid	
At -193.26°C	14.81 dynes/cm
At -206.95°C	18.85 dynes/cm
Heat of transition, solid	173.90 cal/g-mole
Heat of fusion	121.98 cal/g-mole
Heat of vaporization at bp	1561.3 cal/g-mole
Viscosity, liquid	
At -187.96°C	0.257 cP
At -203.96°C	0.414 cP
Viscosity, vapor	
At 0°C, 760 mm Hg	0.0218 cP
Dielectric constant	
At -189.95°C	1.517 ε
At -215.76°C	1.567 ε
Thermal conductivity, gas	
At 0°C, 760 mm Hg	5.92×10^{-5} cal/(sec)(cm^2)(°C/cm)

Source: Adapted from Neumark and Siegmund, 1966, Table 1, p. 506. Reprinted by permission of the publisher.

2.2.1.2 Chemical Properties — Although the effective nuclear charge of the fluorine atom is not as great as that for some other elements, the effective surface charge density of the fluorine atoms is greater than that of any other element because of its small radius (Durrant and Durrant, 1962). As a result, fluorine is the most electronegative and reactive of all elements (Weast, 1978).

The standard oxidation potential for the reaction

$$2F^- \rightleftarrows F_2 + 2e$$

is -2.85 V vs the normal hydrogen electrode (Horton, 1961). Fluorine combines directly at ordinary or elevated temperatures with all elements other than oxygen and nitrogen (Banks and Goldwhite, 1966). When finely dispersed, water, glass, ceramics, carbon, and metals all burn in fluorine with a bright flame (Weast, 1978). Fluorine reacts with water to form hydrogen fluoride and oxygen difluoride, with nitric acid to yield the explosive gas fluorine nitrate, and with sulfuric acid to form fluorosulfuric acid. It reacts violently with most organic molecules, usually with dissociation (Merck, 1976).

Because of the low quantum number and the simple electronic configuration of the fluorine atom, its ability to form diverse bond formations is restricted; the only common states for the fluorine atom in combination are the ionic form, F^-, and the covalent tetrahedral form (Durrant and Durrant, 1962). Some characteristics of these forms are described below.

2.2.1.2.1 Ionic fluorides — Fluorine reacts with metallic elements to form compounds which are usually ionic, both in the crystalline state and in solution; they have characteristically high melting and boiling points (Table 2.2). Most of these salts are readily soluble in water; however, lithium, aluminum, strontium, barium, magnesium, calcium, and manganese fluorides are sparingly soluble or insoluble (Latimer and Hildebrand, 1951). The poor solubility of the last three salts has important physiological consequences in cases of acute fluorosis.

TABLE 2.2. PROPERTIES OF SOME IONIC FLUORIDES

Substance	Formula	Color	Melting point (°C)	Boiling point (°C)	Density, 25°C (g/ml)	Solubility in water, 18°C (g/100 ml)
Aluminum fluoride	AlF_3	White	1040	1291	3.07	0.559 (25°C)
Calcium fluoride	CaF_2	White	1360	2500	3.18	0.0016
Cesium fluoride	CsF	White	682	1251	4.115	367
Lithium fluoride	LiF	White	842	1676	2.601	0.133 (25°C)
Magnesium fluoride	MgF_2	White	1225	2260	3.0	0.0076
Manganese fluoride	MnF_2	Red	856		3.98	0.66 (40°C)
Potassium fluoride	KF	White	846	1505	2.48	92.3
Rubidium fluoride	RbF	White	775	1410	3.557	130.6
Sodium fluoride	NaF	White	988	1695	2.558	4.22
Sodium fluoroaluminate	Na_3AlF_6	White	1000		2.90	0.034 (15°C)
Sodium fluorosilicate	Na_2SiF_6	White	Decomposes		2.679	0.652 (17°C)
Uranium tetrafluoride	UF_4	Green	960		6.7	Slightly soluble

Source: Compiled from Horton, 1961, and Weast, 1978.

Fluoride ion also has a strong tendency to form complexes with heavy polyvalent cations in aqueous solution (Horton, 1961; Thomas and Alther, 1966):

$$Fe^{3+} + 6F^- \rightarrow FeF_6^{3-} \ ,$$

$$Al^{3+} + 6F^- \rightarrow AlF_6^{3-} \ ,$$

$$Mn^{3+} + 5F^- \rightarrow MnF_5^{2-} \ ,$$

$$Mn^{2+} + 3F^- \rightarrow MnF_3^{2-} \ .$$

This behavior, and the previously mentioned formation of insoluble fluorides, are chiefly responsible for the biological toxicity of inorganic fluorides.

2.2.1.2.2 Covalent fluorides — Fluorine and hydrogen fluoride react with nonmetallic elements to form covalent compounds, such as hydrogen fluoride, fluorine monoxide, silicon tetrafluoride, sulfur hexafluoride, organic compounds containing fluorine, and complex anionic forms. Because of the small size of the fluorine atom and its high electronegativity, most elements exhibit their highest oxidation state as fluorides. When all valence-shell electrons of such atoms form covalent bonds with fluorine, increase in coordination number by the formation of ionic crystals is impossible. Crystals of such compounds consist of simple molecules held together by van der Waals forces. Accordingly, in contrast to ionic fluoride compounds, covalent compounds of fluorine tend to have low melting points and high volatility (Durrant and Durrant, 1962). The melting points, boiling points, and other physical properties of a wide variety of covalent fluorides are given in Table 2.3.

2.2.1.3 Occurrence and Synthesis — Because of its great reactivity, elemental fluorine does not usually occur free in the environment and, when introduced, does not long persist unreacted; its occurrence is soon marked by the formation of fluorinated reaction products.

Elemental fluorine is produced on a commercial scale by the electrolysis of a molten solution of potassium fluoride in anhydrous hydrogen fluoride. Fluorine gas is formed at the anode, and hydrogen is formed at the cathode:

$$2HF \rightarrow H_2 + F_2 \ .$$

Some fluorocarbons are also produced during the electrolysis because the anode is made of carbon; however, the main impurity in the product is normally hydrogen fluoride. The latter is removed by passing the product through absorption towers packed with sodium fluoride (National Academy of Sciences, 1971).

2.2.2 Hydrogen Fluoride

First prepared in the anhydrous state by Fremy in 1856 (Gall, 1966), hydrogen fluoride (HF) is now a widely used industrial chemical. Because of this extensive use, hydrogen fluoride is probably the greatest single atmospheric fluoride contaminant. Due to its great reactivity, however, hydrogen fluoride is unlikely to remain in its original form very long.

2.2.2.1 Physical Properties — Hydrogen fluoride is a colorless, pungent liquid or gas that boils at 19.5°C and freezes near -83°C (Weast, 1978). Its heat of formation and energy of dissociation are 64.5 and 140 kcal/mole respectively (Nikolaev et al., 1972). Hydrogen fluoride is highly soluble in water and alcohol and fumes strongly in contact with the atmosphere.

TABLE 2.3. PROPERTIES OF SOME COVALENT FLUORIDES

Substance	Formula	Color	Melting point (°C)	Boiling point (°C)	Density (g/ml)	Solubility in water (g/100 ml)
Ammonium fluoride	NH_4F	White	[a]		1.009 (25°C)	100 (0°C)
Chlorine trifluoride	ClF_3	Colorless	-82.6	12.0	2.04 (-50°C)	Reacts
Dichlorodifluoromethane	CCl_2F_2	Colorless	-158	-30	1.75 (-115°C)	Soluble
Disulfur decafluoride	S_2F_{10}	Colorless	-92	29	2.08 (0°C)	Reacts
Fluoroacetic acid	FCH_2COOH	Colorless	35.2	165	1.369 (36°C)	Soluble
Hexafluoropropene	C_3F_6	Colorless	-156.2	-29.4	1.583 (-40°C)	
Hydrogen fluoride	HF	Colorless	-83.1	19.5	1.0015 (0°C)	Miscible
Oxygen difluoride	OF_2	Colorless	-223.8	-144.8	1.90 (-233°C)	Slightly soluble, reacts
Silicon tetrafluoride	SiF_4	Colorless	-90	-86	1.66 (-95°C)	Reacts
Sulfur hexafluoride	SF_6	Colorless	-50.4	-65[a]	1.88 (-50°C)	Slightly soluble
Sulfur tetrafluoride	SF_4	Colorless	-125.0	-40.0		Reacts
Tetrafluoroethylene	C_2F_4	Colorless	-142.5	-76.3	1.519 (-76°C)	Insoluble
Tetrafluoromethane	CF_4	Colorless	-150	-129	3.034 (0°C)	Slightly soluble
Trichlorofluoromethane	CCl_3F	Colorless	-111	23.7	1.49 (17°C)	Insoluble
Uranium hexafluoride	UF_6	Colorless	69.5	56[a]	4.68 (21°C)	Reacts
Vinyl fluoride	C_2H_3F	Colorless	-160.5	-72.2		Insoluble

[a] Sublimes.

Source: Compiled from Weast, 1978; Horton, 1961; and Merck, 1976.

The density of the liquid is 1.0015 at 0°C (Horton, 1961). The dielectric constant of liquid hydrogen fluoride is near 84 at 0°C; accordingly, its solvent power rivals that of water (Moeller, 1952). The formula weight of hydrogen fluoride is 20.006, but hydrogen bonding between molecules causes a high degree of association, and both the liquid and gas show large departures from ideal behavior (Gall, 1966). Other physical properties of hydrogen fluoride are listed in Table 2.4.

TABLE 2.4. SOME PHYSICAL PROPERTIES OF ANHYDROUS HYDROGEN FLUORIDE

Property	Value
Formula weight, calculated	20.006
Molecular weight	
Saturated vapor at boiling point (bp)	78.24
Saturated vapor at 100°C	49.08
Boiling point at 1 atm	19.51°C
Melting point (mp)	-83.37°C
Density	
Liquid at 25°C	0.9576 g/cm^3
Vapor, saturated, at 25°C	3.553 g/liter
Vapor pressure at 25°C	17.8 psia
Heat of vaporization	
bp at 1 atm	1609 cal/20.01 g[a] 1785[b]
Heat of fusion at mp	46.93 cal/g
Heat capacity at constant pressure	
Liquid at bp	12.2 cal/(20.01 g)(°C)
Vapor at 25°C, 1 atm	143 cal/(20.01 g)(°C)
Heat of formation, ideal gas at 25°C	-64.9 kcal/20.01 g
Free energy of formation, ideal gas at 25°C	-65.0 kcal/20.01 g
Entropy, ideal gas, at 25°C	41.5 cal/(20.01 g)(°C)
Critical temperature	188°C
Critical pressure	941 psia
Critical density	0.29 g/cm^3
Viscosity at 0°C	0.26 cP
Surface tension at bp	8.6 dynes/cm
Refractive index, 5893 Å, at 25°C	1.1574
Molar refractivity, 5893 Å, formula weight	2.13 cm^3
Conductivity at 0°C	$<1.6 \times 10^{-6}$ mho/cm
Dielectric constant at 0°C	83.6
Dipole moment, HF molecule	1.83 D

[a] From vapor pressure vs temperature.
[b] From calorimetry.

Source: Adapted from Gall, 1966, Table 1, p. 611. Reprinted by permission of the publisher.

2.2.2.2 Chemical Properties — Anhydrous hydrogen fluoride is one of the most acidic substances known (Horton, 1961). It readily protonates and dissolves even nonbasic compounds such as alcohols, ketones, and mineral acids:

$$2HF + ROH \rightarrow ROH_2^+ + HF_2^- ,$$

$$2HF + R_2CO \rightarrow R_2COH^+ + HF_2^- ,$$

$$2HF + HNO_3 \rightarrow H_2NO_3^+ + HF_2^- .$$

Fluorides of the alkali metals, silver, barium, ammonia, and thallium dissolve in anhydrous hydrogen fluoride and behave as strong electrolytes. Other metal salts, such as the halides, cyanides, and azides, react with anhydrous hydrogen fluoride to form the fluoride and the corresponding acid. The latter is insoluble in hydrogen fluoride and is expelled (Banks and Goldwhite, 1966):

$$NaCl + HF \rightarrow NaF + HCl .$$

Anhydrous hydrogen fluoride reacts with metals more positive than hydrogen in the electromotive series, providing they do not form insoluble fluoride films, as aluminum does. Anhydrous hydrogen fluoride is also a strong dehydrating agent; wood and paper are charred on contact, and aldehydes undergo condensation by elimination of water (Gall, 1966).

In dilute aqueous solution, hydrogen fluoride is a weak acid; only about 10% is ionized in 0.1 *M* solutions. This behavior is unusual compared with the other hydrogen halides and is attributed to the high bond strength in hydrogen fluoride (Banks and Goldwhite, 1966) (bond energies: hydrogen fluoride, 135; hydrogen chloride, 103; hydrogen bromide, 87; hydrogen iodide, 71 kcal/mole). Aqueous hydrogen fluoride (hydrofluoric acid) is best known for its ability to dissolve glass:

$$4HF + SiO_2 \rightarrow SiF_4 + 2H_2O .$$

Anhydrous hydrogen fluoride, both gaseous and liquid, and higher concentrations of aqueous hydrofluoric acid are all very corrosive to skin, eyes, lungs, and mucous membrane; extreme care should be taken to avoid contact with these materials. With dilute solutions of hydrofluoric acid, burns of the skin may not be evident when exposure occurs but may later become apparent by deep-seated ulceration (Gall, 1966).

2.2.2.3 Synthesis and Use — Based on quantity of production, hydrogen fluoride is the most important manufactured compound of fluorine; it is used directly or as an intermediate in the preparation of almost every fluoride-containing product (Gall, 1966). Hydrogen fluoride is made industrially by treating the mineral fluorspar with concentrated sulfuric acid:

$$CaF_2 + H_2SO_4 \rightarrow CaSO_4 + 2HF \; .$$

The volatile gas is condensed, purified by distillation, and stored in steel tanks and cylinders (Gall, 1966). About 292,000 metric tons were produced in the United States in 1977, primarily as anhydrous liquid or gas (*Chemical Marketing Reporter*, 1978*b*). Approximately 40% of this total was used to manufacture aluminum, and 37% was converted into fluorocarbon compounds and products. The remainder was used chiefly in processing uranium (7%), in alkylation catalysts in petroleum refining (5%), in manufacturing fluoride salts (4%), and in stainless steel pickling operations (4%). Smaller quantities were used as fluxes in metal casting, welding, and brazing operations; etching agents in the glass and ceramics industries; cleaners in metal finishing processes; pesticides; and, of course, for fluoridation of water, toothpaste, and other products.

2.2.3 Fluorspar, Cryolite, and Fluorapatite

The principal fluoride-containing minerals are fluorspar (CaF_2), cryolite ($3NaF \cdot AlF_3$), and fluorapatite [$CaF_2 \cdot 3Ca_3(PO_4)_2$]. Their theoretical fluoride contents are 48.5%, 54.5%, and 3.8% respectively. Commercially, fluorspar is the most important mineral with workable deposits occurring in all major countries. World production in 1975 exceeded 4,150,000 metric tons (Quan, 1976). Almost 29% of this amount (1.2 million metric tons) was consumed in the United States. Physical properties of the purified form of the mineral are listed in Table 2.5. Cryolite is a relatively rare mineral which is an essential raw material in the aluminum industry; its chief physical characteristics are given in Table 2.6. Commercially important deposits of this material were found only in Greenland, and these are now exhausted; present supplies are prepared synthetically (National Academy of Sciences, 1971). Fluorapatite is a constituent of rock phosphate; it contains only a small percentage of fluoride and is presently unimportant as a commercial source of fluorine. Rock phosphate has great environmental significance, however, as well as potential commercial importance in the future, because it is mined and consumed in vast quantities in the production of elemental phosphorus and phosphate fertilizers. Wood (1975) estimated the fluorine content of the phosphate rock mined annually in the United States as 729,000 metric tons. It is estimated that all forms of fluoride emissions to the atmosphere from phosphorus and phosphate fertilizer production in the United States amounted to more than 16,300 metric tons in 1968 (National Academy of Sciences, 1971, p. 9).

Fluorspar, cryolite, and fluorapatite are essentially insoluble in water; accordingly, the mammalian toxicity of these compounds is moderate to low. Their toxicity toward insects is much more pronounced, however, and synthetic cryolite is sometimes used as an insecticide for the control of chewing insects, such as the codling moth, the Mexican bean beetle, flea beetles, and tomato worms (Metcalf, 1966).

TABLE 2.5. PHYSICAL PROPERTIES OF CALCIUM FLUORIDE

Property	Value
Melting point (mp)	1402°C
Boiling point (bp)	2513°C
Heat of fusion	5.5 kcal/mole
Heat of vaporization at bp	80 kcal/mole
Vapor pressure at 2100°C	7.6 mm
Specific heat at constant pressure	
Solid at 25°C	16.02 cal/(mole)(°C)
Solid at mp	30.0 cal/(mole)(°C)
Liquid at mp	23.9 cal/(mole)(°C)
Entropy at 25°C	16.46 cal/(mole)(°C)
Heat of formation, solid at 25°C	-290.3 kcal/mole
Free energy of formation, solid at 25°C	-278.1 kcal/mole
Thermal conductivity, crystal at 25°C	0.0262 cal/sec(cm^2)(°C/cm)
Density	
Solid at 25°C	3.181 g/cm^3
Liquid at mp	2.52 g/cm^3
Thermal expansion, average, at 25°C to 300°C	22.3×10^{-6}/°C
Compressibility at 25°C, 1 atm	1.24×10^{-6}/atm
Hardness	
Mohs scale	4
Knoop, 500-g load	158
Solubility in water	
At 25°C	0.0017 g/100 g water
At 175°C	0.0018 g/100 g water
Refractive index at 24°C, 5893 Å	1.43382
Dielectric constant at 30°C	6.64
Electrical conductivity	
Solid at 20°C	1.3×10^{-18} mho/cm
Solid at 650°C	6×10^{-5} mho/cm
Solid at mp	3.45 mho/cm

Source: Adapted from Gall, 1966, Table 1, p. 574. Reprinted by permission of the publisher.

TABLE 2.6. PHYSICAL PROPERTIES OF CRYOLITE

Property	Value
Formula weight	209.94
Composition, calculated	
Fluorine	54.30%
Aluminum	12.85%
Sodium	32.85%
Melting point (mp)	1009
Transition temperature	
Monoclinic to cubic	560°C
Second-order	881°C
Vapor pressure, liquid, at 1009°C	1.9 mm Hg
Heat of fusion at 1009°C	26.7 kcal/mole
Heat of vaporization at 1009°C	54
Heat of transition	
Monoclinic to cubic at 560°C	2.22 kcal/mole
Second-order at 880°C	<0.2 kcal/mole
Heat capacity	
Monoclinic crystal at 25°C	51.6 cal/(mole)(°C)
Cubic crystal at 560°C	65.0 cal/(mole)(°C)
Liquid at 1009°C	96.8 cal/(mole)(°C)
Entropy, monoclinic crystal at 25°C	57.0 cal/(mole)(°C)
Heat of formation, monoclinic crystal at 25°C	-788.9 kcal/mole
Free energy of formation, monoclinic crystal at 25°C	-750.1 kcal/mole
Density	
Monoclinic crystal at 25°C	2.97 g/cm^3
Cubic crystal from x-ray data, at 560°C	2.77 g/cm^3
Solid at 1009°C	2.62 g/cm^3
Liquid at 1009°C	2.087 g/cm^3
Hardness, Mohs scale	2.5
Optical properties	
Refractive index	
Alpha form	1.3385
Beta form	1.3389
Gamma form	1.3396
Biaxial character	Positive
Axial plane	Perpendicular to (010)
Angle of acute bisectrix to C axis	43°54'
Electrical conductivity	
Liquid at 1009°C	2.83 Ω^{-1} cm^{-1}
Solid at 400°C	4.0×10^{-6} Ω^{-1} cm^{-1}
Viscosity, liquid, at 1009°C	6.7 cP
Surface tension, liquid in air, at 1019°C	125 dynes/cm
Solubility in water	
At 25°C	0.042 g/100 g
At 100°C	0.135 g/100 g

Source: Adapted from Gall, 1966, Table 2, p. 536. Reprinted by permission of the publisher.

2.2.4 Alkali Fluorides

The alkali fluorides have the formula MF, where M represents lithium, sodium, potassium, rubidium, or cesium. The alkali fluorides are typical salts; they have high melting and boiling points and, except for the lithium and sodium compounds, are fairly soluble in water. Other physical properties are listed in Table 2.2. All of the fluorides, except lithium, absorb hydrogen fluoride to give acid fluorides of the type MHF_2 (Banks and Goldwhite, 1966). This reaction is reversible for the sodium and potassium salts; on strong heating they dissociate to yield the normal salt (Durrant and Durrant, 1962):

$$KHF_2 = KF + HF .$$

This reaction is often used to produce small quantities of anhydrous hydrogen fluoride in the laboratory.

Sodium fluoride is the most important member of the alkali fluoride group. It is a white crystalline powder that melts at 988°C and boils at 1695°C (Weast, 1978). It is prepared by neutralizing aqueous hydrofluoric acid with sodium carbonate. The commercial product is only 90% to 95% pure because of the presence of fluorosilicates and other impurities derived from the aqueous hydrofluoric acid (Merck, 1976).

Sodium fluoride is widely used in fluxes, for fluoridation of water supplies, and for scrubbing hydrogen fluoride from fluorine; it is also occasionally used as an insecticide and a wood preservative (Rudge, 1962).

Documentation of fatalities caused by accidental fluoride poisonings shows that sodium fluoride, more than any other compound, is the toxic agent (Eagers, 1969). To prevent the confusion of sodium fluoride with common edible materials such as flour, powdered milk, and baking powder, some states require insecticidal grades of sodium fluoride to be tinted blue green (Merck, 1976).

2.2.5 Silicon Tetrafluoride and Fluorosilicic Acid

Silicon tetrafluoride is a colorless gas that melts at -90°C, boils at -86°C (Weast, 1978), and has a pungent odor reminiscent of hydrogen chloride (Merck, 1976); it is very toxic (Roholm, 1938). Other properties are listed in Tables 2.7 and 2.8. The gas has little utilitarian value; its environmental significance is due to its formation in large quantities during the combustion of coal and the manufacture of normal and triple superphosphate fertilizers, elemental phosphorus, wet-process phosphoric acid, aluminum metal, and brick and tile products. The total fluoride emission to the atmosphere in the United States in 1968 from the above sources, was estimated to be nearly 63,500 metric tons (National Academy of Sciences, 1971). The exact fraction of the total attributed to silicon tetrafluoride is difficult to determine with certainty, but silicon tetrafluoride is known to be the chief gaseous pollutant in many of the manufacturing steps. For example, normal and triple superphosphate fertilizers are prepared by treating rock phosphate with sulfuric acid and phosphoric acid respectively:

$$CaF_2 \cdot 3Ca_3(PO_4)_2 + 7H_2SO_4 + 3H_2O \rightarrow 3CaH_4(PO_4)_2 \cdot H_2O + 7CaSO_4 + 2HF$$

and

$$CaF_2 \cdot 3Ca_3(PO_4)_2 + 14H_3PO_4 + 10H_2O \rightarrow 10CaH_4(PO_4)_2 \cdot H_2O + 2HF \ .$$

In each instance, the resulting hydrofluoric acid reacts further with silica in the rock phosphate to produce silicon tetrafluoride:

$$4HF + SiO_2 \rightarrow SiF_4 + 2H_2O \ .$$

In modern production plants, off-gases are scrubbed with water, and most of the silicon tetrafluoride is removed as fluorosilicic acid (Banks and Goldwhite, 1966):

$$3SiF_4 + 2H_2O \rightarrow 2H_2SiF_6 + SiO_2 \ .$$

However, large quantities of silicon tetrafluoride and hydrogen fluoride escape to the atmosphere in older manufacturing plants.

TABLE 2.7. SOME PHYSICAL PROPERTIES OF SILICON TETRAFLUORIDE

Property	Value
Melting point (triple point), 1318 mm	-90.2°C
Boiling point	-95.0°C
Density of gas at 0°C and 1 atm	4.69 g/liter
Molar refraction	8.40
Heat of capacity at constant pressure	18.2 cal/(mole)(°C)
Critical temperature	14.15 ± 0.02°C
Critical pressure	36.66 ± 0.05 atm
Heat of formation at 25°C	-370 kcal/g-mole
Heat of sublimation at 177.7°K and 1 atm	6.15 kcal/g-mole
Heat of fusion at 182.9°K and 1320 mm	1.69 kcal/g-mole
Heat of vaporization at 182.9°K and 1320 mm	4.46 kcal/g-mole
Heat of dissociation	232 kcal/g-mole
Vapor pressure, log p, mm	8.453-957.0/T
Sublimation pressure, log p, mm	10.469-1352.8/T

Source: Adapted from Byrns, 1966, Table 1, p. 651. Reprinted by permission of the publisher.

TABLE 2.8. SOLUBILITY OF SILICON TETRAFLUORIDE IN VARIOUS SOLVENTS

Solvent	Silicon tetrafluoride content (g/100 g solvent)
Acetic acid	1.1
Acetone	3.1
Butyl alcohol	23.4
Ethyl alcohol, absolute	36.4
Ethyl alcohol, 95%	38.1
Glycerol	5.7
Glycol	26.2
Isopropyl alcohol	28.2
Methanol	32.8

Source: Adapted from Byrns, 1966, Table 2, p. 651. Reprinted by permission of the publisher.

Fluorosilicic acid (H_2SiF_6), which is formed from the reaction of silicon tetrafluoride and water, is extremely toxic (Waldbott, 1963). The acid is very water-soluble and is readily absorbed by vegetation. Concentrated solutions are corrosive to glass, ceramics, some metals, and metal oxides (National Academy of Sciences, 1971). Fluorosilicic acid is sometimes used in hardening cement, preserving timber, manufacturing enamels, and preserving oil pigments (Merck, 1976). Small amounts of the sodium salt of fluorosilicic acid (Na_2SiF_6) are used as an insecticide; it is said to be more potent as an insecticide and less toxic to higher animals than sodium fluoride (Metcalf, 1966; Rudge, 1962). The physical properties of some inorganic fluorosilicates are shown in Table 2.9.

2.2.6 Halogen Fluorides

Fluorine reacts with other halogens to form the interhalogen compounds shown in Table 2.10. Although some interhalogen compounds have been known for well over a hundred years, those containing fluorine are relatively new and are still the objects of considerable research (Emeléus, 1969).

The halogen fluorides are very active compounds that react with most metals and nonmetals as vigorously as elemental fluorine (Banks and Goldwhite, 1966); consequently, they are not normally present in the environment. They are occasionally released, however, in chemical laboratories and in certain rocket-engine test firings (National Academy of Sciences, 1971). Such gases are rapidly hydrolyzed by moisture in the air to form hydrogen fluoride, oxygen, and other products.

Halogen fluorides are synthesized in the laboratory by direct union of the elements, variations in reaction conditions determining the particular species formed when alternative combinations are possible. Chlorine

TABLE 2.9. PHYSICAL PROPERTIES OF SOME INORGANIC FLUOROSILICATES

Fluorosilicate	Formula	Appearance	Specific gravity	Refractive index		Solubility in water (g/100 ml)	Solubility	
				ω	ε		In alcohol	In acetone
Aluminum	$Al_2(SiF_6)_3 \cdot 9H_2O$	Hexagonal prisms				Very soluble (cold)		
Ammonium	$(NH_4)_2SiF_6$	Colorless, cubic	2.011	1.3692		18.58 (17.5°C)	Soluble	Insoluble
Barium	$BaSiF_6$	Colorless, rhombic needles	4.279			0.025 (25°C)	Insoluble	Insoluble
Calcium	$CaSiF_6 \cdot 2H_2O$	White, tetragonal	2.662			10.58 (22°C), decomposes	Soluble	
Cupric	$CuSiF_6 \cdot 6H_2O$	Blue, rhombic, efflorescent	2.270	1.4092	1.4080	233 (17°C)	Slightly soluble	
Ferrous	$FeSiF_6 \cdot 6H_2O$	Pale blue green, trigonal	1.961	1.3638	1.3848	128.2	Insoluble	Insoluble
Lithium	$Li_2SiF_6 \cdot 2H_2O$	White, monoclinic	2.33	1.300	1.296	73 (17°C)	Soluble	Insoluble
Potassium	K_2SiF_6	Colorless, cubic	2.746	1.339		0.177 (25°C)	Insoluble	
Sodium	Na_2SiF_6	Colorless, hexagonal	2.755	1.312	1.309	0.762 (25°C)	Insoluble	

Source: Adapted from Byrns, 1966, Table 3, p. 658. Reprinted by permission of the publisher.

TABLE 2.10. HALOGEN FLUORIDES: TYPES AND BOILING POINTS

AB	AB_3	AB_5	AB_7
ClF (-101°C)	ClF_3 (12°C)	ClF_5	IF_7 (277°C, sublimes)
BrF (20°C)	BrF_3 (126°C)	BrF_5 (41°C) IF_5 (101°C)	

Source: Adapted from Emeléus, 1969, p. 11. Reprinted by permission of the publisher.

trifluoride (ClF_3) is probably the most frequently used halogen fluoride. It is colorless as a gas, yellow green as a liquid, and white as a solid. Chlorine trifluoride boils near 12°C and melts at -83°C; its odor is sweet and suffocating. The specific gravity of chlorine trifluoride is 1.82 at 20°C (Bryce, 1964). Glass wool and organic matter burst into flames on contact with chlorine trifluoride, and even quartz is attacked if traces of moisture are present. Chlorine trifluoride is used in nuclear reactor fuel processing, as a fluorinating agent in chemical reactions, and as an igniter and propellant for rockets (Merck, 1976).

2.2.7 Group VIA Fluorides

Fluorine reacts with the Group VIA elements to form a variety of fluorides. Most of these compounds are infrequent contaminants of the environment. Only the following compounds are of interest here: oxygen difluoride, sulfur tetrafluoride, disulfur decafluoride, and sulfur hexafluoride.

Oxygen difluoride (OF_2) is a toxic, colorless gas sometimes used as a high-energy oxidizer in rocket-propulsion systems. Oxygen difluoride can also occur in laboratory or manufacturing operations when fluorine reacts with dilute aqueous sodium hydroxide solutions (Emeléus, 1969) or when halogen fluorides are hydrolyzed (National Academy of Sciences, 1971). Oxygen difluoride melts at -223.8°C and boils at -145.3°C (Streng, 1963). It does not attack glass in the cold, reacts only slowly with water, but corrodes mercury. Unlike fluorine and hydrogen fluoride, oxygen difluoride is not readily detectable by smell and causes no immediate discomfort even in potentially lethal concentrations; however, it is considered the most dangerous of the gaseous inorganic fluorine compounds (Lester and Adams, 1965). Other properties of oxygen difluoride are listed in Table 2.11.

Sulfur tetrafluoride (SF_4) is a colorless gas that reacts violently with water and attacks glass but not quartz or mercury. It melts at -121°C, boils at -40.4°C, and is thermostable to 600°C; other physical properties are given in Table 2.12. It is prepared on an industrial scale by treating sodium fluoride with sulfur dichloride in acetonitrile at 70 to 80°C (Merck, 1976). Sulfur tetrafluoride is a unique fluorinating agent for organic compounds; it directly replaces carbonyl oxygen with fluorine. Accordingly, sulfur tetrafluoride is mainly used in the laboratory and

TABLE 2.11. SOME PHYSICAL PROPERTIES OF OXYGEN DIFLUORIDE

Property	Value
Formula	OF_2
Formula weight	54.0
Color	Colorless (gas), pale yellow (liquid)
Freezing point	-223.8°C
Boiling point	-145.3°C
Critical temperature	-58.0°C
Critical pressure	48.9 atm
Critical density	0.553 g/ml
Heat of formation	7.6 kcal/mole
Heat of vaporization	2.65 kcal/mole
Dipole moment	0.1759 D
Density	
Gas, normal temperature and pressure	2.41 mg/ml
Liquid at melting point	1.932 g/ml

Source: Adapted from Streng, 1963, Table I, pp. 608-609. Reprinted by permission of the publisher.

TABLE 2.12. SELECTED PHYSICAL PROPERTIES OF SULFUR TETRAFLUORIDE

Property	Value
Melting point (mp)	-121°C
Boiling point (bp)	-40.4°C
Critical temperature	90.9°C
Surface tension at bp	19.85 dynes/cm
Dipole moment	0.632 D
Heat of formation	-171.7 ± 2.5 kcal/g-mole
Molar heat of vaporization	6320 cal
Trouton's constant	27.1

Source: Adapted from Brown, 1966, Table 5, p. 671. Reprinted by permission of the publisher.

chemical manufacturing operations to prepare *gem*-difluorides from aldehydes and ketones and to convert carboxylic acids to trifluoromethyl compounds (Banks and Goldwhite, 1966). Sulfur tetrafluoride is very poisonous; its toxicity is comparable with that of phosgene (Merck, 1976).

Disulfur decafluoride (S_2F_{10}) is a dense colorless liquid that melts at -92°C and boils at 29°C (Weast, 1978). Other physical properties are listed in Table 2.13. Disulfur decafluoride occurs as a toxic by-product

TABLE 2.13. SOME PHYSICAL PROPERTIES OF DISULFUR DECAFLUORIDE

Property	Value
Melting point	-92°C
Boiling point	29°C
Trouton's constant	23.0
Surface tension at 0°C	13.9 dynes/cm
Liquid density at 0°C	2.08 g/ml
Specific electrical conductivity	10^{-13} mho/ml
Dielectric constant at 10°C	2.030
Heat of vaporization	7 kcal/g-mole

Source: Adapted from Brown, 1966, Table 6, p. 673. Reprinted by permission of the publisher.

when sulfur is treated with fluorine; it can also be formed by the partial decomposition of sulfur hexafluoride in high-voltage electric discharges (National Academy of Sciences, 1971). Disulfur decafluoride is very reactive and is considered more toxic than fluorine (Eagers, 1969).

Unlike sulfur tetrafluoride and disulfur decafluoride, sulfur hexafluoride (SF_6) is nontoxic and chemically inert (Banks and Goldwhite, 1966). It is prepared by burning sulfur in fluorine and washing the product with aqueous alkali to remove the unwanted tetrafluorides and decafluorides. The colorless gas, which melts at -50.4°C and sublimes at -65°C under 2.21 atm absolute pressure, is widely used as a dielectric in high-voltage electrical equipment (National Academy of Sciences, 1971). Several additional properties of this compound are listed in Table 2.14.

2.2.8 Organic Fluorides

Covalently bound fluorine so closely resembles hydrogen that it is possible, in principle, to synthesize fluorine analogs for almost all of the presently known hydrocarbons and their derivatives; already, several thousand fluorine-containing organic compounds have been prepared (Banks and Goldwhite, 1966). The chemical and physiological properties of many of these compounds differ greatly from those of their hydrocarbon counterparts. These differences stem largely from the greater electronegativity of fluorine, compared with hydrogen, which makes the covalent carbon-fluorine bond much stronger (approximately 116 kcal/mole) than the similar carbon-hydrogen bond (approximately 99 kcal/mole) (Barry and Norbury, 1974). Of the many potentially interesting organic fluorides, this report can consider here only two classes: fluorocarbons and naturally occurring monofluoro aliphatic compounds.

2.2.8.1 Fluorocarbons — Technically, the term fluorocarbon refers to compounds containing only carbon and fluorine. It is used here, however, in its popular sense which encompasses, additionally, compounds containing the other halogens or hydrogen or both.

TABLE 2.14. PROPERTIES OF SULFUR HEXAFLUORIDE

Property	Value
Chemical formula	SF_6
Molecular weight	146.0504
Critical temperature	45.54°C
Critical pressure	37.600×10^5 N/m^2
Critical density	736.0 kg/m^3
Triple-point temperature	-50.0°C
Triple-point pressure	2.3267×10^5 N/m^2
Boiling point at 1.0133×10^5 N/m^2	-68.0°C
Melting point	-50.7°C
Transition temperature	-179.15°C
Density of solid at -91.15°C	2550.0 kg/m^3
Coefficient of thermal expansion of liquid at melting point	2.90×10^{-3} C^{-1}
Heat of formation of gas at 25°C	-1.2217×10^9 J kg-mole^{-1}
Free energy of formation of gas at 25°C	-1.1177×10^9 J kg-mole^{-1}
Heat of sublimation at -63.7°C	2.2860×10^7 J kg-mole^{-1}
Heat of fusion	5.0242×10^6 J kg-mole^{-1}
Heat of transition	1.6077×10^6 J kg-mole^{-1}
Entropy of gas at 25°C	2.9187×10^5 J kg-mole^{-1} C^{-1}
Heat capacity ratio of gas at 25°C and 1.0133×10^5 N/m^2	1.29
Velocity of sound at 0°C and 1.0133×10^5 N/m^2	131.2 m/sec
Dielectric constant of gas at 25°C and 1.0133×10^5 N/m^2	1.002049
Refractive index of gas at 25°C and 1.0133×10^5 N/m^2	1.000766
Threshold limit value	1000 ppm (6×10^{-3} kg/m^3)

Source: Adapted from Horvath, 1975, Table 9.1, p. 118. Reprinted by permission of the publisher.

Fluorocarbons are prepared by various techniques. The most direct method is based on fluorination of a vaporized hydrocarbon using elemental fluorine. The reaction is highly exothermic and must be performed in the presence of inert gas diluent in a metal-packed reactor that can remove excess heat before thermal dissociation of the product occurs. Less energetic reaction conditions are possible in certain instances, by replacing the fluorine gas with less potent fluorinating agents, such as hydrogen fluoride, cobalt(III) fluoride, silver(II) fluoride, antimony(III) fluoride, chlorine monofluoride, or chlorine trifluoride. For some products, the method of choice involves an entirely different technique — the electrolysis between nickel electrodes of a solution of the organic compound in anhydrous hydrogen fluoride. A potential of 5 to 6 V is usually applied.

Hydrogen is evolved at the cathode, and fluorination of the dissolved compound takes place at the anode; no anodic gas is formed. Usually, all hydrogen atoms in the dissolved compound are replaced with fluorine, double bonds are saturated, fragmentation of the molecule occurs, and functional groups are retained. See Banks and Goldwhite (1966), Emeléus (1969), Federal Task Force on Inadvertant Modification of the Stratosphere (1975), Rudge (1962), and Sargent and Seffl (1970) for extensive discussions of these preparatory procedures.

Fluorination of organic compounds and products constituted the greatest single use of hydrogen fluoride in the United States in 1968; about 90,700 metric tons was used that year for that purpose (National Academy of Sciences, 1971). By 1977, U.S. consumption of hydrogen fluoride had grown to 108,000 metric tons, resulting in the production of 386,000 metric tons of fluorocarbons (*Chemical Marketing Reporter*, 1978*a*, 1978*b*). The fluorocarbons, which were used chiefly for aerosol propellants (24%), refrigerants (39%), solvents (11%), and blowing agents (12%), included dichlorodifluoromethane, trichlorofluoromethane, tetrafluoromethane, tetrafluoroethene, vinyl fluoride, and hexafluoropropene. The first three compounds of this group have high chemical stability, low toxicity, low boiling points, and vapor pressures suitable for propellants or refrigerants. The last three compounds are unsaturated and can be polymerized into solids that have exceptionally low coefficients of friction and remarkable chemical inertness. The physical properties of these and related compounds are summarized in Table 2.15. In 1978, nonessential uses of fluorocarbon propellants were banned by the U.S. Environmental Protection Agency, and this segment of the fluorocarbon market shrank to about 2% of its former value. As a consequence, the total U.S. market for fluorocarbons was expected to decline to about 309,000 metric tons in 1978 and perhaps even further in 1979. However, annual increases of 5% to 6% are projected for overall fluorocarbon demand from 1980 through 1983 (*Chemical Marketing Reporter*, 1978*a*).

In addition to the major uses of fluorocarbons cited above, numerous minor applications also exist such as fire extinguishing agents (CF_3Br, CF_2Br_2, and CF_2BrCl), inhalation anesthetics ($CF_3CHBrCl$), and specialized cleaners and degreasers ($CF_2ClCFCl_2$) (Banks and Goldwhite, 1966; Rudge, 1962).

Although saturated compounds of carbon and fluorine are neither toxic nor narcotic, many of the higher unsaturated compounds of carbon and fluorine or of carbon, hydrogen, fluorine, and other halogens are very toxic (Rudge, 1962).

2.2.8.2 Fluoroacetate and Fluorooleic Acid — Thousands of synthetically prepared organic compounds containing fluorine are known, but only a few occur naturally. Historically and physiologically, the most important of these are the monofluoroaliphatic compounds, fluoroacetic acid (FCH_2COOH) and ω-fluorooleic acid [$F(CH_2)_8CH{=}CH(CH_2)_7COOH$(*cis*)]. Fluoroacetic acid is the toxic principle in the leaves of various species of *Dichapetalum*, *Acacia*, *Gastrolobium*, and *Palicourea* (Smith, 1970); it melts at 35.2°C, boils at 165°C, and is soluble in water and alcohol (Weast, 1978). At the pH of cell sap, the compound presumably exists as the fluoroacetate.

TABLE 2.15. SOME PHYSICAL PROPERTIES OF ALIPHATIC FLUOROCARBONS

Compound	Formula	Molecular weight	Boiling point (°C)	Melting point (°C)	Liquid density (g/ml)	Liquid refractive index (n_D)	Critical temperature (°C)	Critical pressure (atm)	Critical volume (ml/g)
Trichlorofluoromethane	CCl_3F	137.38	23.8	-111	1.476 (25°C)	1.374 (25°C)	198.0	43.2	1.804
Dichlorodifluoromethane	CCl_2F_2	120.93	-29.8	-158	1.311 (25°C)	1.287 (25°C)	112.0	40.6	1.792
Chlorotrifluoromethane	$CClF_3$	104.47	-81.4	-181	1.298 (-30°C)	1.199 (-73°C)	28.9	38.2	1.729
Dichlorofluoromethane	$CHCl_2F$	102.93	8.9	-135	1.366 (25°C)	1.354 (25°C)	178.5	51.0	1.917
Chlorodifluoromethane	$CHClF_2$	86.48	-40.8	-160	1.194 (25°C)	1.256 (25°C)	96.0	49.1	1.904
Chlorofluoromethane	CH_2ClF	68.48	-9.1	-133	1.271 (20°C)				
Tetrafluoromethane	CF_4	88.01	-128.0	-184	1.317 (-80°C)	1.151 (-73°C)	-45.7	37.0	1.598
Trifluoromethane	CHF_3	70.02	-82.0	-155	0.670 (25°C)	1.215 (-73°C)	25.9	47.7	1.904
Difluoromethane	CH_2F_2	52.03	-51.7	-136	1.100 (20°C)				
Fluoromethane	CH_3F	34.03	-78.5	-142	0.843 (-60°C)				
Hexafluoroethane	$CF_3{-}CF_3$	138.02	-78.2	-101	1.587 (-73°C)	1.206 (-73°C)	19.7	29.4	1.648
Pentafluoroethane	$CHF_2{-}CF_3$	120.03	-48.5	-103		1.5012 (19°C)			
1,1,1,2-Tetrafluoroethane	$CH_2F{-}CF_3$	102.04	-26.5	-101					
1,1,1-Trifluoroethane	$CH_3{-}CF_3$	84.04	-47.6	-111	0.942 (30°C)				
1,1-Difluoroethane	$CH_3{-}CHF_2$	66.05	-24.7	-117	0.966 (19°C)	1.3011 (-72°C)			
Fluoroethane	$CH_3{-}CH_2F$	48.06	-37.1	-143	0.818 (-37°C)	1.3033 (-37°C)			
Tetrafluoroethene	$CF_2{=}CF_2$	100.02	-76.3	-142.5	1.519 (-76°C)		33.3		
1,1,2-Trifluoroethene	$CHF{=}CF_2$	82.03	-61	-78	1.265 (27°C)	1.3011 (-72°C)			
1,1-Difluoroethene	$CH_2{=}CF_2$	64.04	-85.7		0.750 (10°C)		29.7	44.0	2.415
Fluoroethene (vinyl fluoride)	$CH_2{=}CHF$	46.05	-72.0	-160	0.675 (10°C)		54.7	51.7	3.125
Octafluoropropane	$CF_3{-}CF_2{-}CF_3$	188.03	-36.7	-183	1.350 (20°C)		71.9	26.45	1.592
Hexafluoropropene	$CF_3CF{=}CF_2$	150.02	-29.4	-156.2	1.583 (-40°C)				

Source: Adapted from Downing, 1966, Table 1, p. 740, and Table 4, p. 744. Reprinted by permission of the publisher.

Unlike inorganic fluorides, fluoroacetic acid is not poisonous, per se; it has little or no effect on isolated enzymes or those organized in mitochondria (Bartlett and Barron, 1947). However, fluoroacetic acid is readily metabolized to fluorocitric acid. One of the isomers of fluorocitric acid irreversibly complexes aconitase which prevents the conversion of citric acid to aconitate, disrupts the Krebs cycle, and causes metabolic death of the cell (Goldman, 1969). Fluoroethanol (FCH_2CH_2OH), which also is not intrinsically toxic, has an apparent odor of toxicity as great as fluoroacetate because of its metabolic conversion to the latter (Pattison and Peters, 1966). The sodium salt of synthetically produced fluoroacetic acid was formerly used extensively in the United States for the control of rats and other mammalian pests ("rodenticide 1080"). However, its manufacture and use in interstate commerce for predator control was banned by the U.S. Environmental Protection Agency (1972). Accidental ingestion of this compound has resulted in several human fatalities (Sollmann, 1957) (see Section 6).

ω-Fluorooleic acid occurs in the seeds of *Dichapetalum toxicarium*, along with traces of lower ω-fluorocarboxylic acids containing an even number of carbon atoms in their chain. The presence of these trace compounds suggests that ω-fluorooleic acid is built up in steps of two carbon atoms from the lower fatty acids, beginning with fluoroacetate (Pattison and Peters, 1966). Like fluoroacetate, ω-fluorooleic acid inhibits aconitase in kidney mitochondrial preparations, causes citrate accumulation in the tissues, and is toxic to mammals. Although the lower odd-numbered carbon acids show little or no toxicity, all the lower even-numbered carbon acids are poisonous. These circumstances correspond to the Knoop rule for the degradation of fatty acids and suggest that the toxicity of the even-numbered carbon compounds is due to their ultimate degradation to fluoroacetate (Pattison, 1959).

2.2.9 Uranium Hexafluoride

In the United States, isotopically enriched uranium required for nuclear reactor fuel and nuclear weapons is obtained by processing uranium hexafluoride in gaseous diffusion plants (Drury, 1967, p. 85). Preparation of the necessary uranium hexafluoride requires large amounts of hydrogen fluoride; in 1974 about 9000 metric tons was used for this purpose (*Chemical and Engineering News*, 1975).

Uranium hexafluoride can be prepared from naturally occurring uranium oxides by a variety of processes; the method used for large-scale production is dictated by economic considerations. Currently, a series of chemical steps are employed. The trioxide is first reduced to the dioxide by treatment with hydrogen gas at 700°C:

$$UO_3 + H_2 \rightarrow UO_2 + H_2O \ .$$

The resulting dioxide is converted to the tetrafluoride with hydrogen fluoride at 500°C:

$$UO_2 + 4HF \rightarrow UF_4 + 2H_2O \ .$$

The latter is then converted to the final product by treatment with fluorine at 350°C:

$$UF_4 + F_2 \rightarrow UF_6 \ .$$

The intermediate product, uranium tetrafluoride, is a green crystalline solid that melts at 960°C. It is only poorly soluble in water and is relatively inert chemically (Simons, 1964).

Uranium hexafluoride is a colorless, extremely corrosive, crystalline solid that sublimes at 56°C and melts at 69.2°C under 2 atm pressure (Rudge, 1962). Other physical properties are listed in Table 2.16. Uranium hexafluoride is a very reactive, toxic compound, a powerful oxidizer, and a strong fluorinating agent (Banks and Goldwhite, 1966; Eagers, 1969). Uranium hexafluoride attacks glass and quartz, but not copper, nickel, aluminum, or silver; it is also unreactive to oxygen, nitrogen, chlorine, bromine, and saturated fluorocarbons (Simons, 1964).

TABLE 2.16. SOME PHYSICAL PROPERTIES OF URANIUM HEXAFLUORIDE

Formula	UF_6
Formula weight	352.07 g
Color	Colorless to pale yellow
Melting point	69.5°C
Boiling point	56°C (sublimes)
Density	4.68 g/ml at 21°C
Dipole moment	0.0 D
Dielectric constant	$4.2 \times 10^{-3}\ \varepsilon^{-1}$
Surface tension	15.6 dynes/cm at 80°C
Heat of formation	
Solid	-516 kcal/mole at 25°C
Heat of vaporization	6.907 kcal/mole at 64°C
Heat of fusion	4.588 kcal/mole at 64°C
Heat of sublimation	11.495 kcal/mole at 64°C

Source: Compiled from Horton, 1961, and Mattson, 1970.

Although large quantities of uranium hexafluoride are handled at atomic energy installations and industrial plants, stringent control measures are applied because of the radioactivity of the uranium, and as a result few emissions to the environment occur (National Academy of Sciences, 1971).

2.3 ANALYSIS FOR FLUORIDE

2.3.1 Sampling and Sample Preparation

Fluorides occur in the environment as organic and inorganic gases, liquids, and solids. Specialized techniques are required to obtain representative samples of the various forms, and certain precautions are necessary to avoid contamination of, or loss of fluoride from, these samples. The principal requirements for handling the various sample types are discussed in the following sections.

2.3.1.1 Fluoride in Air — Hydrogen fluoride, silicon tetrafluoride, and particulates such as sodium and calcium fluorides constitute the chief inorganic fluoride contaminants of air (National Academy of Sciences, 1971). Fluorine, because of its great reactivity, is rarely present in elemental form. Concentrations of fluoride in excess of 1.5 $\mu g/m^3$ seldom occur in nonindustrial atmospheres; the usual range is from less than 0.05 to 0.5 $\mu g/m^3$ (Katz, 1968; Thompson, McMullen, and Morgan, 1971). Higher concentrations are found near facilities manufacturing steel, aluminum, phosphorus, phosphate fertilizers, bricks, pottery, and ceramics, as well as near large coal-fired power plants. Samples may be collected manually or automatically. Manual techniques include sorption of fluorides on beads or tubing coated with sodium bicarbonate or other basis compounds, or use of a liquid-filled bubbler (Figure 2.1) or impinger-type collector (Figure 2.2) with or without a prefilter to remove particulate fluorides (Jacobson and Weinstein, 1977). Countercurrent scrubbers and spray columns are also utilized. Usually, at least 1 to 10 m^3 of air are processed. Bubblers and scrubbers normally contain water or a solution of sodium or potassium hydroxide; the resulting fluoride solution may be further processed, prior to analysis, as decribed in Section 2.3.1.2. Automated equipment for determining gaseous fluorides in air are also available. These instruments extract gaseous fluoride constituents from air with absorbing liquids or solids during a specified sampling period, then flush the sample into a cell where the fluoride ion concentration is measured by a specific ion electrode whose signal is amplified and displayed on a recording device (Jacobson and Weinstein, 1977). Particulate fluorides are prefiltered and are not determined in presently available instruments. When it is important to determine particulate fractions of the sample, fractionation is usually accomplished by prefiltering the air sample (Mandl et al., 1971; Pack et al., 1959). The particulate matter generally requires fusion with alkali hydroxide to convert it into a soluble form prior to separating the fluoride for analysis (Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969). Sampling devices and procedures are discussed in detail by the American Industrial Hygiene Association (1972), Hendrickson (1968), Israel (1974), Jacobson and Weinstein (1977), MacDonald (1970), Marshall and Wood (1968), and Thompson, McMullen, and Morgan (1971).

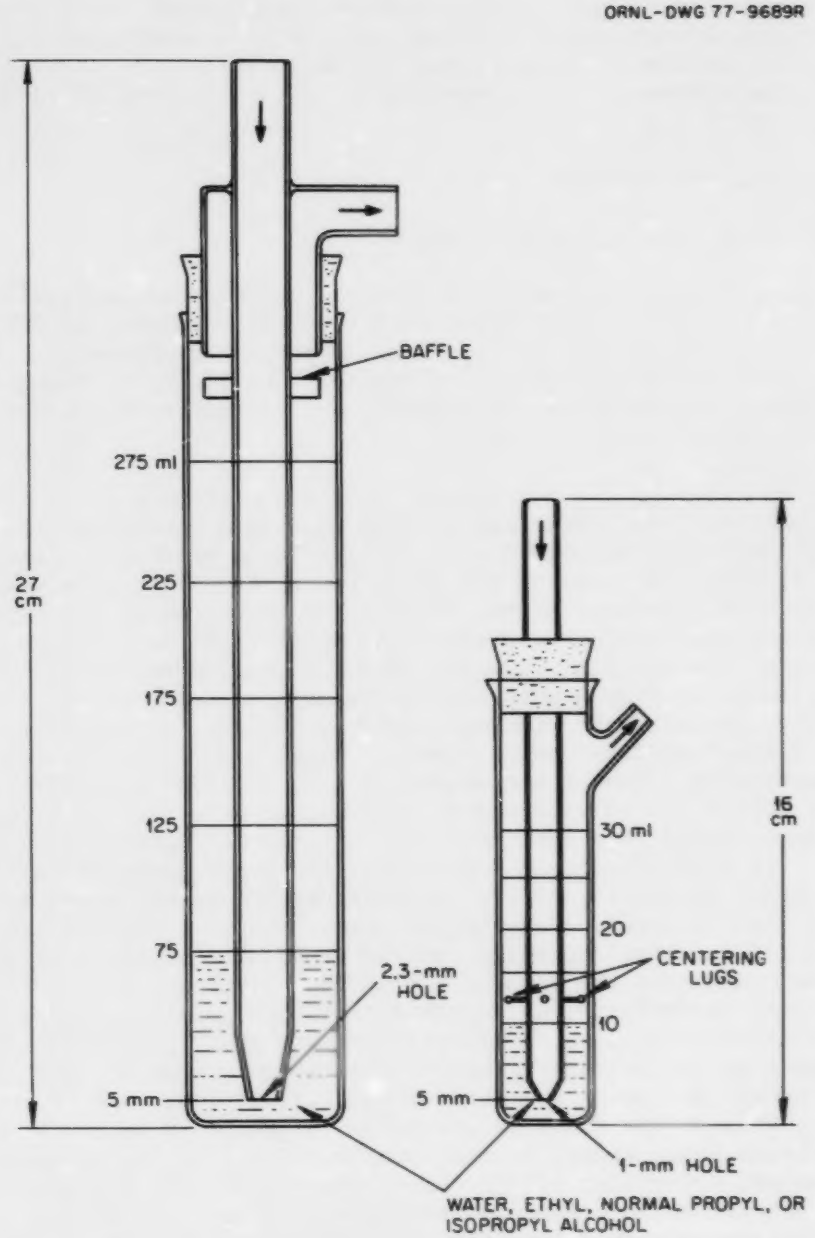

ORNL-DWG 77-9689R
BAFFLE
275 ml
225
175
125
75
27 cm
2.3-mm HOLE
5 mm
16 cm
30 ml
20
CENTERING LUGS
10
5 mm
1-mm HOLE
WATER, ETHYL, NORMAL PROPYL, OR ISOPROPYL ALCOHOL

Figure 2.1. Typical wet-impinger collector. Source: Adapted from American Industrial Hygiene Association, 1972, Figure 9-22, p. 133. Reprinted by permission of the publisher.

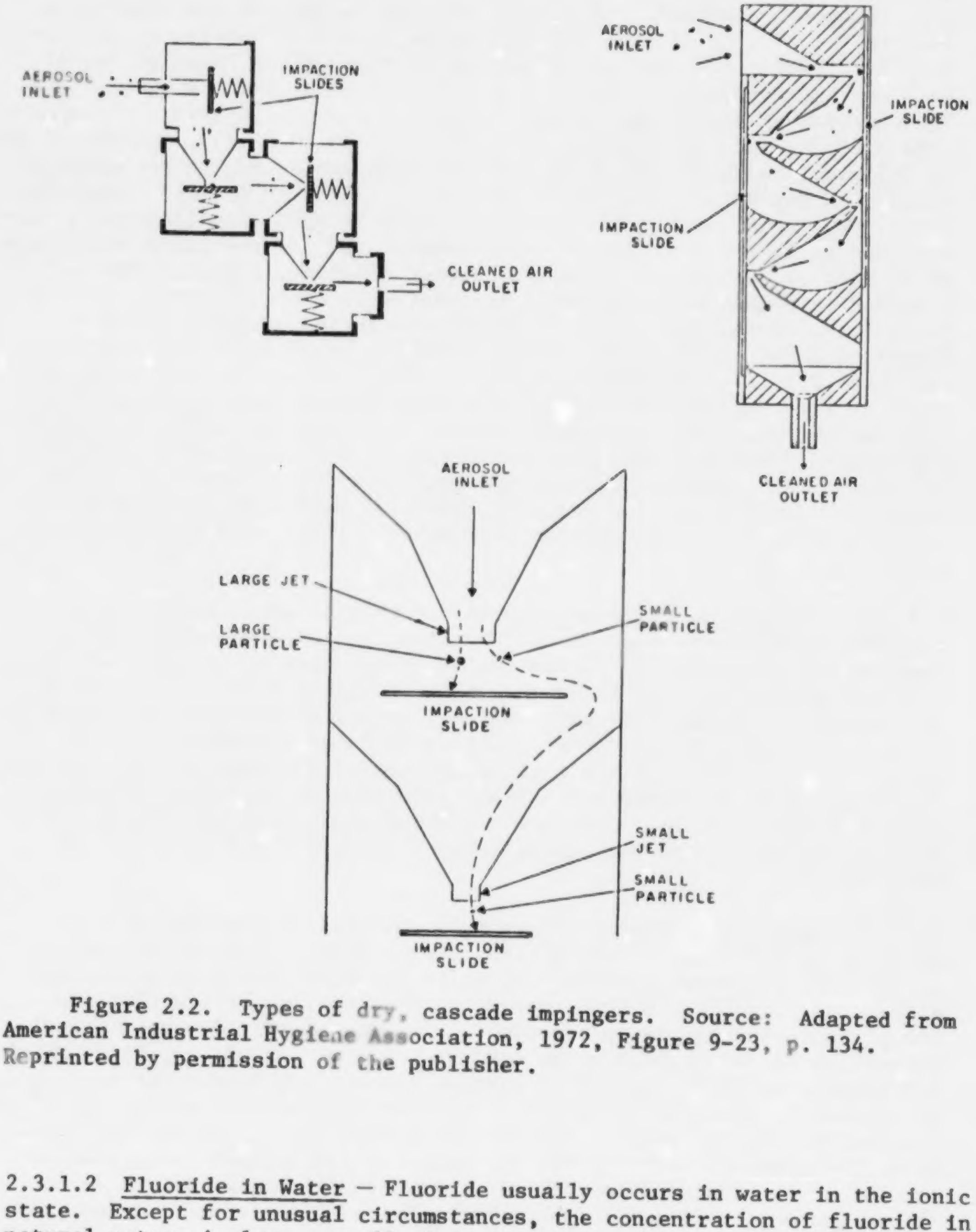

ORNL-DWG 77-12899
AEROSOL INLET
IMPACTION SLIDES
CLEANED AIR OUTLET
AEROSOL INLET
IMPACTION SLIDE
IMPACTION SLIDE
CLEANED AIR OUTLET
AEROSOL INLET
LARGE JET
LARGE PARTICLE
SMALL PARTICLE
IMPACTION SLIDE
SMALL JET
SMALL PARTICLE
IMPACTION SLIDE

Figure 2.2. Types of dry, cascade impingers. Source: Adapted from American Industrial Hygiene Association, 1972, Figure 9-23, p. 134. Reprinted by permission of the publisher.

2.3.1.2 Fluoride in Water — Fluoride usually occurs in water in the ionic state. Except for unusual circumstances, the concentration of fluoride in natural waters is low — usually less than 1 ppm (Cholak, 1959*a*). However, the concentration of fluoride in industrial waste streams depends on the chemical process involved; for example, typical processing solutions from the chemical milling of titanium metal contain about 100,000 ppm fluoride (Staebler, 1975). Samples are normally taken in glass or, preferably, polyethylene containers. Typically, storage losses are not troublesome (Sholtes et al., 1973), but acidic samples should be handled in plastic or other inert containers and should be treated with a known excess of base or fixative (Horton, 1961). When interfering ions are absent, the fluoride determination can be made directly; however, polyvalent complexable cations, such as aluminum, silicon(IV), and iron(III), interfere; so do chloride, sulfate, and phosphate (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971). For colorimetric analysis a preliminary acid distillation is recommended to free the sample of contaminants. If the resulting distillate is colored or turbid, the solution is made alkaline, the fluoride is adsorbed on magnesium oxide which is removed by centrifugation, and the preliminary distillation step is repeated with the purified product (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971). When fluoride is determined by the electrode technique, the distillation step can usually be eliminated [even when the sample contains such ions as aluminum, hexametaphosphate, iron(III), and orthophosphate] if a citrate buffer solution is added to the sample. The citrate complexes the interfering cations, releasing fluoride ion for analysis (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971).

2.3.1.3 Fluoride in Soils and Minerals — This class of samples often requires extensive preanalysis preparation, and careful manipulation is required to avoid loss or contamination of the sample. Samples are usually homogenized in ball or hammer mills. Organic matter, if present, is removed by ashing. For soil samples, ashing is normally performed at about 575°C, using fluoride-free calcium oxide as a fixative (Horton, 1961). Refractory materials that do not easily dissolve in sulfuric or perchloric acid are fused with alkali carbonate or hydroxide to convert them to a soluble form. Jacobson and Weinstein (1977) and Brewer (1965) reviewed in detail the various aspects of preparing soil and mineral samples.

2.3.1.4 Fluoride in Plant Tissues — Representative sampling of plant material is difficult to accomplish. Variations in composition occur, not only in different parts of the plant but also diurnally and seasonally. Additionally, it is necessary to distinguish surface fluoride on the plant from fluoride in the plant. In general, field errors greatly exceed laboratory errors in the analysis of plant materials; only by taking relatively large numbers of samples can the population variability be adequately measured (Allen et al., 1974; Jacobson and Weinstein, 1977). When collected, the samples are usually washed, dried, ashed, and fused with alkali before the fluoride is separated for analysis. If samples are not to be processed immediately, they are frequently stored in a frozen state with fluoride-free calcium oxide added to prevent loss of fluoride.

Various washing techniques are used. The Intersociety Committee on Methods for Ambient Air Sampling and Analysis (1969) recommends a gentle 30-sec bath in a polyethylene vessel containing a solution 0.05% in Alconox and 0.05% in sodium ethylenediaminetetraacetate, followed by 10-sec rinses in each of three beakers of deionized water. Typically, drying is performed in an oven at 70 to 80°C for 24 to 48 hr, usually without a lime fixative. Ashing is accomplished by heating the plant material in a nickel, Inconel, or platinum crucible to 400 to 600°C for 2 hr. In many plant samples this ashing step results in refractory fluorides that do not readily dissolve in subsequent operations; it is therefore good technique to fuse the ashed sample with alkali carbonate or hydroxide to ensure solubility of the residue (National Academy of Sciences, 1971). After fusion, the melt is dissolved and the fluoride is separated for analysis. Also see Jacobson and McCune (1969), Jacobson and Weinstein (1977), and Kakabadse et al. (1971) for additional discussion of sample handling techniques.

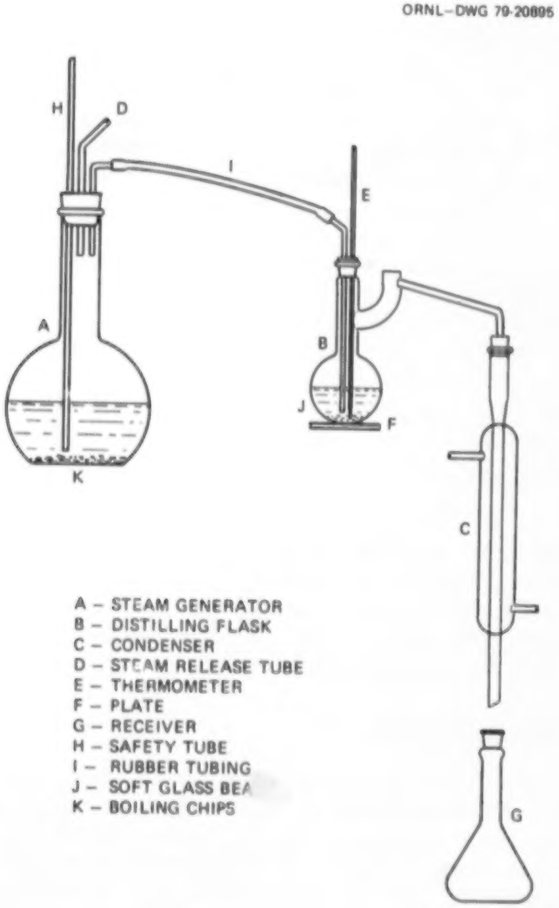
2.3.1.5 Fluoride in Animal Tissues — The procedures described for plant samples are generally applicable to animal tissues, except that rapid freezing prior to storage is desirable because of the lower stability of animal tissues. Also, since most soft tissues contain little silica or aluminum, they seldom form refractory fluorides, and fusing the ashes of these tissues with alkali carbonate or hydroxide is generally unnecessary. Samples of skeletal tissues are simply prepared; they are freed from flesh, dried, and ashed at 500 to 600°C. The fluoride in the residue is then ready for separation and analysis. After gentle evaporation to dryness, body fluids (e.g., blood, serum, and urine) are treated similarly (National Academy of Sciences, 1971); however, this procedure is not always necessary if the specific ion electrode or diffusion methods are used to determine fluoride concentrations. Venkateswarlu (1975) compared ten different procedures for determining fluoride in unwashed bovine and human sera; he found results for nonionic fluoride and total fluoride erratic, especially by diffusion methods, and recommended ashing such samples prior to analysis.

Reagents commonly used in large amounts in preparing plant and animal tissue for analysis, such as alkaline fixatives, often contain sufficient fluoride to alter the subsequent analysis drastically. If realiable assays are to be obtained, the utmost caution must be exercised to use fluoride-free materials (Weddle and Maurer, 1954).

2.3.2 Separation of Fluoride

Substances are present in many analytical samples that interfere in the subsequent determination of fluoride. Accordingly, except for specific ion electrode analysis, it is common practice to isolate the fluoride from other constituents of analytical samples (Horton, 1961; Jacobson and Weinstein, 1977). Distillation, diffusion, ion exchange, and precipitation techniques are most frequently used.

2.3.2.1 Distillation — Until recently, distillation was the most widely used technique for separating fluoride from other constituents of analytical samples (Jacobson and Weinstein, 1977; MacDonald, 1970; National Academy of Sciences, 1971; Nikolaev et al., 1972). Many variants of the method exist, but the basic procedure (Willard and Winter, 1933) consists of volatilizing hexafluorosilicic acid from an acid solution in the presence of glass or quartz, with steam vapor as the carrier. A representative distillation apparatus is shown in Figure 2.3. Recovery of fluoride depends on the geometry of the container, temperature, acid, and impurities; generally, distillation from perchloric acid at 135°C is preferred. Chloride in the distillate interferes but can be fixed by addition of a soluble silver salt. Similarly, small amounts of interfering sulfate can be fixed with a soluble barium salt; however, excess barium must be avoided since insoluble barium fluoride may be precipitated (Horton, 1961).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H
D
I
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A – STEAM GENERATOR
B – DISTILLING FLASK
C – CONDENSER
D – STEAM RELEASE TUBE
E – THERMOMETER
F – PLATE
G – RECEIVER
H – SAFETY TUBE
I – RUBBER TUBING
J – SOFT GLASS BEA
K – BOILING CHIPS

Figure 2.3. Apparatus for distillation of fluoride. Source: Adapted from Katz, 1977, Figure 203.1, p. 388. Reprinted by permission of the publisher.

2.3.2.2 Diffusion — The isolation of fluoride in microsamples is often accomplished by the diffusion method (National Academy of Sciences, 1971). In this simple technique, an aliquot of the prepared sample is mixed with acid and sealed in a vessel containing an alkali, which absorbs the liberated hydrogen fluoride gas. Typically, the sample aliquot contains 30 μg of fluoride, or less, in a volume of 1 ml. It is placed in a polyethylene container (Conway diffusion dish) and mixed with 2 ml of concentrated perchloric acid. The microdiffusion dish is then quickly sealed with a lid on whose inner surface 0.05 ml of alcoholic sodium hydroxide solution has been deposited and dried. The sealed dish is heated in a 60°C oven for 16 to 20 hr, after which the alkaline absorbent is removed for fluoride analysis by the desired analytical method. An alternative arrangement is shown in Figure 2.4. Interfering materials that volatilize under the described conditions must be fixed or eliminated prior to the diffusion process. Sulfites are converted to nonvolatile sulfates by treatment with hydrogen peroxide, and chlorides are fixed by adding silver perchlorate (Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969, p. 72). Because the required diffusing time increases with fluoride concentration, this method of isolating fluoride from the sample matrix is only used for samples containing small amounts of fluoride (Horton, 1961). Obviously, only acid-labile fluoride is collected in the diffusion method. If other forms of fluoride are present, previously described preanalysis procedures must be used to convert them to the acid-labile form. See Jacobson and Weinstein (1977), MacDonald (1970), Nicholson (1966), Singer and Armstrong (1965), and Taves (1968*b*) for further discussion of the separation of fluoride by the diffusion technique.

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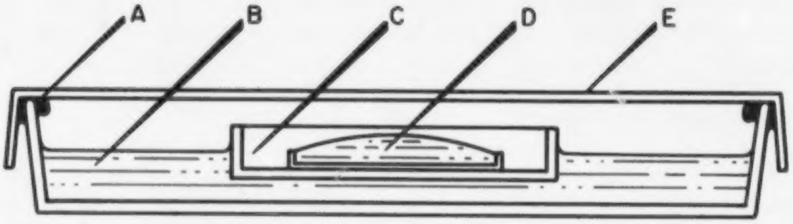

Figure 2.4. Diffusion apparatus: (*a*) vaseline seal, (*b*) acidified sample, (*c*) plastic cup, (*d*) trapping solution, and (*e*) lid. Source: Adapted from Taves, 1968*b*, Figure 1, p. 970. Reprinted by permission of the publisher.

2.3.2.3 Ion Exchange — Samples may be freed of cationic contaminants by preferentially absorbing the fluoride species on an ion exchange resin, followed by desorption in a small volume of eluant (Jacobson and Weinstein, 1977; MacDonald, 1970; Nielsen, 1960; Nikolaev et al., 1972). A typical chromatographic column consists of a borosilicate tube that has an inside diameter of 10 mm and is 16 cm long, with a fritted glass disk and a stopcock sealed in the base and a 100-ml reservoir attached to the top. The column is filled to a height of 10 to 12 cm with preconditioned 60 to 100 mesh anion exchange resin, such as Duolite A-41, Ionac A 302, Permutit A, or Rexyn 205. Fluoride is fixed on the resin when the acidified sample is passed through the column; interfering cations are not absorbed and are discarded. The fluoride is eluted from the resin with 0.1 *N* sodium hydroxide, and the purified fluoride solution is analyzed by a convenient method (Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969). Ion exchange methods are also useful for concentrating dilute solutions of fluoride. Despite its usefulness, however, the ion exchange method of separating fluoride appears to be little used, compared with the distillation and diffusion techniques.

2.3.2.4 Precipitation — In general, fluoride compounds are more soluble than salts conventionally used as precipitates in analytical chemistry (Nikolaev et al., 1972); consequently, precipitation is now used only infrequently to isolate fluoride from other elements in the sample. When the technique is used, the precipitated species is usually calcium fluoride or lead chlorofluoride (Horton, 1961; MacDonald, 1970; McKenna, 1951*a*).

2.3.2.5 Solvent Extraction — Venkateswarlu (1974) isolated and concentrated fluoride from a variety of biological materials by equilibrating acidified samples with diphenylsilanediol dissolved in toluene; the fluoride is extracted into the immiscible organic solvent as fluorosilane. The latter is then back-extracted into an aqueous sodium hydroxide solution as fluoride ion, which can be analyzed with the fluoride ion electrode or other convenient technique. Good recoveries are obtained, and a 30- to 50-fold increase in fluoride concentration is possible by reducing the volume of the back extract. However, separation of fluoride by solvent extraction sometimes yields negatively biased results (Jacobson and Weinstein, 1977), and the technique does not appear to be used extensively.

2.3.3 Methods of Analysis

Fluoride in environmental samples can be determined by a variety of procedures; those which are currently important or show promise of future usefulness are described in this section. The performance and limitations of each method are emphasized rather than minute operational details. Summaries of the methods are given in Table 2.17. It is important to realize that variations in sensitivity, precision, and accuracy occur, not only among different methods but also among various models of analytical instruments and among different operators (Karasek, 1975): the tabulated data should therefore be considered representative rather than definitive. Performance data obtained from sources interested in developing a new method usually reflect optimized conditions. Interlaboratory comparisons, when they exist, offer more realistic comparisons of the various methods.

TABLE 2.17. METHODS FOR DETERMINING FLUORIDE

	Analytical method		
	Spectrophotometry	Fluoride ion electrode	Titrimetry
Important application	Samples from air, water, soil, and biological sources	Natural fresh and saline waters, industrial waste solutions, atmospheric gases, mists and particulates, soils and minerals, biological fluids and solids	Minerals, water, air, urine, and blood
Limit of detection	0.2 μg/ml (zirconyl-SPADNS, zirconyl eriochrome, cyanine R)[a] 0.015 μg/ml (lanthanum alizarin complexone)[a]	0.04 ng/ml (rainwater)[b] 40 μg/g (rock)[c] 0.25 ng/g (air)[d] 0.1 μg/g (water)[e]	5 to 50 μg[a]
Precision (relative standard deviation)	8.0% (830 μg/liter, zirconyl-SPADNS, no interferants)[f] 12.8% (680 μg/liter, zirconyl-SPADNS, with interferants)[f]	2.9% (900 μg/liter) (water)[f] 3.4% (1.7 μg/g) (standard solution)[d] 1.0% (684 μg/liter) (seawater)[h] 9.8% (94 ng/g) (rabbit plasma)[i]	5% to 10% (30 μg)[g]
Accuracy (relative error)	1.2% (830 μg/liter, zirconyl-SPADNS, no interferants)[f] 5.9% (680 μg/liter, zirconyl-SPADNS, with interferants)[f]	4.9% (900 μg/liter) (water)[f] 2.9% (1.7 μg/g) (standard solution)[d] 0.9% (684 μg/liter) (seawater)[h] 2.6% (94 ng/g) (rabbit plasma)[i]	1% (1 μg/g)[j] 10% to 20% (50 μg)[k]
Interfering substances	Aluminum, iron(III), silicon(IV), chloride, sulfate, and phosphate	Extremes of pH and polyvalent cations, such as silicon(IV), iron(III), and aluminum	Aluminum, barium, calcium, iron(III), thorium, titanyl, vanadyl, zirconium, phosphate, and sulfate ions
Selectivity	Ionic fluoride is determined.	Only ionic fluoride is determined.	Only fluoride is determined.
Comments	Used extensively in field applications and is well seasoned	Rapidly becoming the method of choice for virtually all types of fluoride samples	Older method largely superseded by more recent techniques

[a] Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969.
[b] Warner and Bressan, 1973.
[c] Ficklin, 1970.
[d] Elfers and Decker, 1968.
[e] U.S. Environmental Protection Agency, 1974.
[f] American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971.
[g] Hardin, 1953.
[h] Warner, 1971.
[i] Hall et al., 1972.
[j] McKenna, 1951*a* and 1951*b*.
[k] Nikolaev et al., 1972.

2.3.3.1 Spectrophotometry — This analytical method is based on the increased or decreased absorbance of monochromatic light by a metal-dye complex when the metal ion of the dye reacts with fluoride. The amount of absorbed light is measured with a spectrophotometer and is compared with a previously determined calibration plot that relates light absorption to the concentration of fluoride in the sample. Various absorption complexes are used by different analysts; those most commonly used are the zirconyl alizarin, the zirconyl-SPADNS or the zirconyl Eriochrome Cyanine R compounds, and the lanthanum or cerium alizarin complexones (Jacobson and Weinstein, 1977; MacDonald, 1970; National Academy of Sciences, 1971). The three complexes first mentioned are highly colored initially but are decolorized by reaction with fluoride; the initially red complexones are converted to new, stable, blue-colored forms in the presence of fluoride ion. The effective range of the zirconyl-SPADNS and the zirconyl Eriochrome Cyanine R systems is 0.00 to 1.40 μg of fluoride per milliliter, and the detection limit is 0.02 μg/ml. For the lanthanum alizarin complexone, these quantities are 0.00 to 0.5 μg of fluoride per milliliter and 0.015 μg/ml respectively (Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969, p. 79). In a comparison involving 53 laboratories, a synthetic unknown sample containing 830 μg/liter of fluoride and no interfering ions was determined without distillation by the SPADNS method with a relative standard deviation of 8.0% and a relative error of 1.2%. With distillation, the relative standard deviation increased to 11.0% and the relative error to 2.4%. In a similar synthetic sample that contained appreciable quantities of interfering constituents, the 53 laboratories determined fluoride with a relative standard deviation of 12.8% and a relative error of 5.9% (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971). In general, aluminum, iron, phosphate, and sulfate interfere, and their effects must be eliminated by one of the preanalysis treatments discussed in Section 2.3.2.

The level of precision attainable by the various modifications of the spectrophotometric method is adequate for most environmental and industrial applications; consequently, it has been widely used for the analysis of samples derived from air, water, soil, and biological sources (Ashley, 1960; Bethea, 1974; Hargreaves, Ingram, and Cox, 1970; Megregian, 1954). Now, however, it is being replaced by the more convenient fluoride ion electrode technique (Jacobson and Weinstein, 1977; MacDonald, 1970).

2.3.3.2 Fluoride Ion Electrode — Fluoride ion concentrations can be rapidly and precisely determined in a wide variety of samples, often without elaborate sample preparation, by means of the fluoride ion electrode. The fluoride ion electrode (Figure 2.5) is somewhat similar to the familiar glass pH electrode, except that the membrane is a disk of single-crystal rare earth fluoride, such as lanthanum, praseodymium, or neodymium fluoride (Frant and Ross, 1966). The electrode is filled with a solution containing both fluoride and chloride ions, and an electrical contact is provided by a silver-silver chloride wire; it is used with a pH meter and an external reference electrode, such as the standard saturated potassium chloride-calomel type (Figure 2.6). The crystal membrane of the fluoride ion electrode is appreciably permeable only to the fluoride ions, and the cell potential follows the Nernst equation relationship

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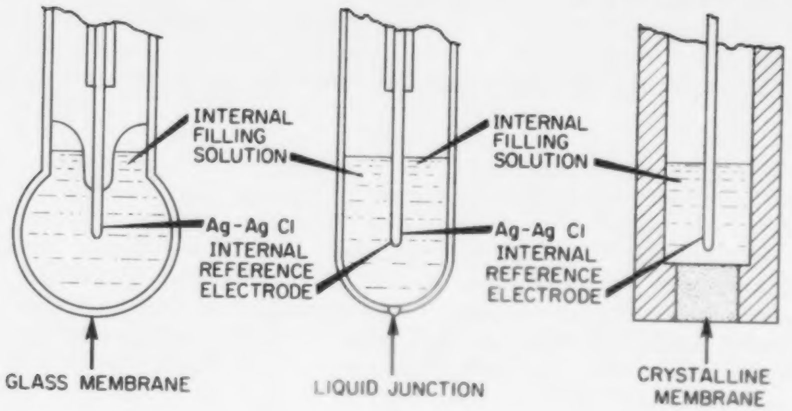
INTERNAL
FILLING
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INTERNAL
REFERENCE
ELECTRODE
GLASS MEMBRANE
LIQUID JUNCTION
CRYSTALLINE
MEMBRANE

Figure 2.5. Cross sections of typical selective ion electrodes. Source: Babcock and Johnson, 1968, Figure 1, p. 954. Reprinted from JOURNAL American Water Works Association Volume 60 by permission of the Association. Copyrighted 1968 by the American Water Works Association, Inc., 6666 W. Quincy Avenue, Denver, Colorado 80235.

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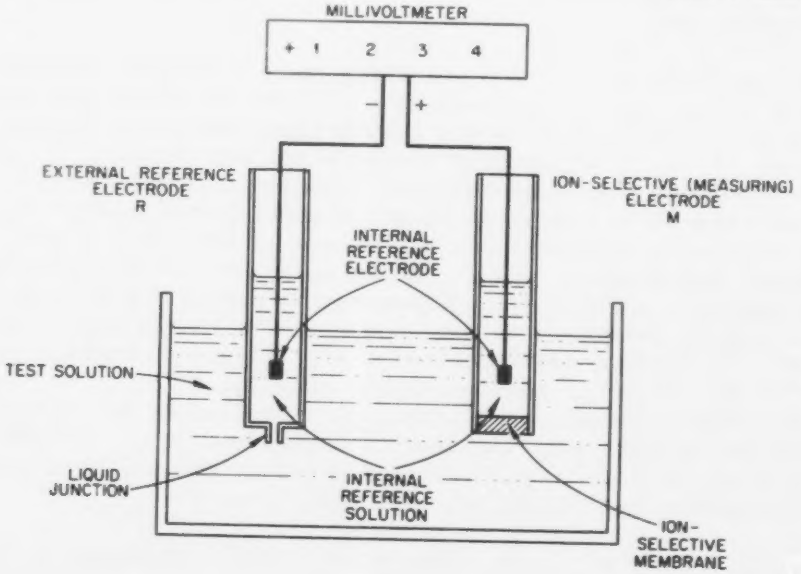
MILLIVOLTMETER
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EXTERNAL REFERENCE
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TEST SOLUTION
LIQUID
JUNCTION
INTERNAL
REFERENCE
SOLUTION
ION-
SELECTIVE
MEMBRANE

Figure 2.6. Potentiometric measuring circuit showing measuring (M) and reference (R) electrodes and potentiometers. Source: Adapted from Light and Cappuccino, 1975, Figure 1, p. 247. Reprinted by permission of the publisher.

over more than five orders of magnitude of fluoride ion activity. The electrode is unresponsive to most other common anions; interference by hydroxide ion occurs, but only at concentrations equal to or greater than the fluoride ion concentration. This interference by hydroxide can be minimized by controlling the pH of the sample; it causes no difficulty at pH 5.

The fluoride electrode measures fluoride activity, rather than concentration; it is therefore necessary to ensure that samples and reference solutions are at equal ionic strengths if measurements interpretable in terms of concentration are to be obtained (Frant and Ross, 1968). The pH and ionic strength requirements of the electrode are satisfied by adding to the samples and standards a high-ionic-strength buffer that also contains citrate ion or other complexing agents to preferentially complex metal ions such as iron or aluminum, and assure that the fluoride in the samples is available in the free, uncomplexed form (Light and Cappuccino, 1975).

The fluoride in a sample is measured by immersing the fluoride and reference electrodes in a solution of the dissolved sample containing the total ionic strength adjustment buffer. When a steady potential is established, a meter reading is made and confirmed; if the method of standard additions is used for calibration (Fuchs et al., 1975; Warner, 1973) additional spikes are added, new readings are made, and the original sample concentration is determined from the response curve. Alternatively, the electrode potential can be converted to the fluoride concentration in the sample by use of standard reference solutions or a previously determined calibration curve.

The range of the fluoride ion electrode is normally considered to be from about 0.2 to 2.0 mg of fluoride per liter (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971); progressively longer times are required to achieve steady readings as the lower concentration limit is approached. However, with proper electrode conditioning and a specialized technique, the lower fluoride concentration limit can be reduced to less than a part per billion (Warner and Bressan, 1973). In an interlaboratory comparison involving 111 analysts, a synthetic unknown sample containing 850 μg of fluoride per liter and no interferants was determined by the electrode method with an average relative standard deviation of 3.6% and an average relative error of 0.7%. A second unknown sample containing 750 μg of fluoride per liter with added phosphate and carbonate interferants was determined by the electrode method with a relative standard deviation of 4.8% and a relative error of 0.2%. Precision and accuracy are adequate for almost all environmental and industrial applications.

Because of its excellent performance, speed, and general convenience, the fluoride ion electrode is rapidly becoming the method of choice for determining fluoride in a wide variety of environmental and industrial samples (Devine and Partington, 1975; Erdmann, 1975; Jacobson and Weinstein, 1977; MacDonald, 1970; Melton, Hoover, and Ayers, 1974; Torma, 1975). See Andelman (1968) for an extensive discussion of the theory and application of ion-selective electrodes in general.

2.3.3.3 Titrimetry — In titrimetric methods, a solution containing fluoride ion and an indicating agent is titrated with a standard solution containing an ion that forms a complex with fluoride. When the end point is reached and all of the fluoride is reacted, additional titrant reacts with the indicator, producing a color change that signals completion of this titration. The quantity of fluoride in the sample is determined from the volume and concentration of the titrant consumed, and the stoichiometry of the reaction. Thorium nitrate is the most commonly used titrant for fluoride ion (Horton, 1961); it is useful in determining microgram to milligram quantities of fluoride. Alizarin Red S is the most widely used indicator for this reaction; it has a light yellow color in dilute acid solutions that contain only alkali metal ions and fluoride ion but forms a pink lake in the presence of uncomplexed thorium. Ions that complex or form insoluble compounds with fluoride or thorium interfere with the titration and must be removed in preliminary treatments. Aluminum, barium, calcium, iron(III), thorium, vanadyl, zirconium, titanyl, phosphate, and sulfate ions are the principal offenders (Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969, p. 74). Zirconium, iron, aluminum, cerium, silver, and uranium(IV) solutions may also be used as titrants (Horton, 1961).

The precision of titrimetric methods varies in different systems; typically, relative standard deviations of 5% to 10% are obtained with samples containing about 30 μg of fluoride (Horton, 1961, p. 260). Relative errors of 1% to 20% are reported for samples containing 1 to 700 μg of fluoride (McKenna, 1951*a*; Nikolaev et al., 1972).

Formerly, titrimetric methods were used extensively for the determination of fluoride in all types of samples. During the last decade, however, these methods have been largely superseded by the spectrophotometric and fluoride ion electrode techniques.

2.3.3.4 Other Methods — Several analytical methods for determining fluoride are not widely used; some have limited applications while others are less convenient, precise, or economical than methods now in use. These methods are briefly described and referenced below.

Gravimetric methods are based on the precipitation of fluoride as the sparingly soluble calcium fluoride or lead chlorofluoride. The dried precipitates are weighed, and the fluoride content of the sample is calculated from the weight and gravimetric factor of the precipitate. The method is useful for fluoride-rich minerals or compounds but is not sufficiently sensitive for general environmental use. McKenna (1951*b*) discussed the technique in detail.

The activity of some enzymes is inhibited by fluoride ion, for example, the conversion of ethyl butyrate to butyric acid by liver esterase. Linde (1951) used this effect to determine fluoride at the microgram level in body fluids by adding liver esterase to the sample, incubating it for 5 hr, and titrating the liberated acid with sodium hydroxide. He observed a sensitivity of about 0.1 μg of fluoride per gram and a reproducibility of about 0.1 to 0.2 μg of fluoride for samples containing 0.5 to 5 μg of fluoride. The method was later adapted to the determination of nanogram quantities of fluoride by McGaughey and Stowell (1964).

Direct polarographic measurement of fluoride is not feasible; however, several cations that form well-defined fluoride complexes (such as aluminum and iron) can be determined by this technique and can provide a basis for indirect determination of fluoride. MacNulty, Reynolds, and Terry (1952) developed such a method based on complexing fluoride with excess aluminum, followed by polarographic determination of the unused aluminum. Concentrations of fluoride as low as 0.2 μg/g were satisfactorily measured. Shoemaker (1955) also developed an indirect method of determining fluoride polarographically, based on the reduction of the diffusion current of a standard iron solution owing to complexation by fluoride ion. The procedure determines 1 μg of fluoride with a relative error of about 15% (also see Gawargious, Besada, and Faltaoos, 1975).

Several fluorometric techniques are based on quenching the fluorescence of metal-dye complexes by fluoride (Horton, 1961; Taves, 1968*a*); although ultrasensitive — their detection limit is about 20 μg of fluoride — they are all seriously affected by extraneous ions and are not used much today.

Fluoride can also be determined by indirect atomic absorption spectrometry techniques. Bond and O'Donnell (1968) developed two such methods. The first is based on depressing the absorption of magnesium in an air-coal-gas flame with fluoride; the second depends on enhancing the absorption of zirconium with fluoride ion. In the absence of interfering ions, the first method is useful over the range of 0.2 to 20 μg of fluoride per milliliter. The second method is applicable to the concentration range from 5 to 200 μg of fluoride per milliliter. Gutsche, Kleinoeder, and Herrmann (1975) also reported a sensitive indirect atomic absorption spectrometric method for fluoride; it is based on reacting excess sodium vapor with the sample and measuring the resulting decrease in atomic sodium concentration. A detection limit of 0.8 μg of fluoride is claimed.

A variety of nuclear activation methods have been developed to determine fluorine. Feldman and Battistone (1966) measured fluoride in bacteriological media by monitoring the 10.7-sec activity from fluorine-20 produced in the $^{19}F(\omega,\gamma)^{20}F$ reaction. The method is applicable to fluoride concentrations down to about 5 μg/ml.

Jeffery and Bakes (1967) analyzed fluorite ores and concentrates by irradiating samples with fast neutrons and measuring the activity of the resulting nitrogen-16. No sample preparation, other than crushing, is required. The technique is not suitable for trace analysis. Ohno et al. (1970) used a photonuclear activation technique to measure fluoride in urine. They observed a detection limit of about 0.01 μg of fluoride and a precision of about 10% in the range from 1 to 2 ppm. Other nuclear activation methods are discussed by Bressan et al. (1974), Fiarman and Schneier (1972), Lauff, Champlin, and Przybylowicz (1973), and Nikolaev et al. (1972).

2.3.4 Comparison of Analytical Procedures

There are many analytical procedures for determining fluoride; the standard technique by which other methods are usually judged consists of isolating the fluoride by a Willard-Winter distillation and analyzing the resulting solution by the zirconium-SPADNS spectrophotometric method. This well-seasoned procedure has a detection limit of about 20 μg of fluoride per milliliter, a characteristic precision of about 10%, and an accuracy of about 5%; it is thus quite adequate for use with most environmental samples (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971; Intersociety Committee on Methods for Ambient Air Sampling and Analysis, 1969: McFarren, Moorman, and Parker, 1969).

Despite the attractive features of the standard zirconium-SPADNS spectrophotometric technique, it is not the method of choice for determining fluoride in most environmental and industrial samples today. That distinction belongs to the fluoride ion electrode method. The popularity and effectiveness of the fluoride ion electrode has steadily increased since it was introduced by Frant and Ross in 1966. Generally speaking, no pretreatment is required for dissolved samples other than the addition of the total-ionic-strength adjustment buffer. However, care is required on this point because the electrode monitors only fluoride ion activity — bound or complexed fluoride is not detected. The normal detection limit of the electrode (about 0.2 mg of fluoride per liter) (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1971) can be extended to less than a part per billion by special procedures (Warner and Bressan, 1973). The precision and accuracy of the electrode method equal or somewhat exceed those of the spectrophotometric or titrimetric techniques for most samples. These factors, along with demonstrated speed and convenience, ensure the future dominance of this method.

In earlier years, titrimetric methods were extensively used for determining fluoride. In capable and experienced hands, some of the procedures, especially the thorium nitrate-alizarin Red S titration, still produce good results. They are, however, a great deal more time consuming than the electrode method and are subject to greater operator variance; consequently, they are infrequently used.

The remaining methods of determining fluoride are mostly specialized procedures that are appropriate for selected samples or that make use of specialized facilities; they seem unlikely to find widespread, generalized application.

SECTION 2

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SECTION 3

BIOLOGICAL ASPECTS IN MICROORGANISMS

3.1 SUMMARY

Some microorganisms are capable of interacting with fluoride-containing pollutants in the environment, altering the form, concentration, and potential hazard of the compounds. The types of fluoride compounds to which microorganisms are exposed are those used in water fluorination, dentifrices, aerosols, pesticides, and compounds associated with industrial air pollution. Fluoride and fluoroorganic compounds are also used as a biochemical tool for investigating microbial metabolism. A few strains of bacteria utilize fluoride compounds as catabolites. Several fluorocarbohydrate intermediates have been isolated. Up to 900 ppm fluoride (47.4 micromoles per gram wet weight) has been reported in lichens and as much as 180 ppm (8.9 micromoles per gram) in oral bacteria. In some cases, fluoride accumulation can be reversed by a recuperation period or by washing the cells.

Toxic and metabolic effects from fluoride exposure are reported for bacteria, yeast, fungi, algae, protozoa, and viruses. Toxic effects include developmental and morphological alterations, growth inhibition, and reduction in infectivity. Dormant bacterial spores are more resistant than vegetative cells to fluoride aerosols; higher molecular weight compounds and compounds containing more chlorine than fluoride seem to be the most toxic to some microorganisms (e.g., *Staphylococcus aureus*). Fluoride acts as a metabolic inhibitor altering such processes as respiration, photosynthesis, carbohydrate metabolism (e.g., sugar uptake, ion transport, acid production, and glycolysis), and enzyme activity. A substrate analog of phenylalanine, *p*-fluorophenylalanine, and an analog of uracil, 5-fluorouracil, can be incorporated into protein and nucleic acids. Some effects of these compounds are: reduction of synthesis of protein, RNA, and DNA; interference with cell differentiation, chromosomal alterations, and changes in ribosomal composition and properties; and modification of messenger RNA functions.

3.2 METABOLISM

Uptake, accumulation, and biotransformation of fluoride and fluoroorganic compounds by microorganisms are the subjects of this section. Studies of microbial metabolism of fluoride compounds have involved several types of organisms under diverse sets of objectives. Strains of bacteria capable of metabolizing fluoride compounds are important because of their possible degradation of fluoride-containing pollutants in streams and soils. Accumulation of fluoride in microorganisms is discussed in terms of uptake in laboratory cultures and in natural environments, including those in human saliva and dental plaque.

3.2.1 Uptake and Accumulation

Several strains of bacteria that can metabolize fluorocarbons have been isolated. From creek water, Kelly (1965) isolated a gram-negative rod (National Collection of Industrial Bacteria No. 9562) capable of growing very slowly with fluoroacetate (40 m*M*) or fluoroacetamide (40 m*M*) as the sole carbon and fuel source. (Fluoroacetamide also served as a nitrogen source.) The growth was linear rather than exponential, suggesting inhibition due to the accumulation of toxic fluoride by-products. Goldman (1965) isolated a pseudomonad from Potomac River mud which used fluoroacetate (50 m*M*) as its sole carbon source, and Goldman, Milne, and Pignataro (1967) isolated *Pseudomonas* species that utilized 2-fluorobenzoic acid as its sole carbon source. A *Pseudomonas* species isolated by Harper and Blakley (1971) used *p*-fluorophenylacetic acid as its sole carbon and fuel source (Figure 3.1). However, a *Vibrio* 01 strain exhibited no growth in 48 hr in a medium containing *p*-fluorobenzoate even when inoculated with cells adapted to benzoate (Ali, Callely, and Hayes, 1962). Bowman et al. (1964) and Bowman and Mallette (1966) found that *Escherichia coli* could degrade *p*-fluorophenylamine by a process that involves an initial deamination; products included a number of fluoride-containing compounds in addition to fluorophenylacetate.

Examples of microbial incorporation of fluoride compounds into protein and nucleic acids are numerous. Effects of such incorporated fluoride are given in Section 3.3.2.3. Fluoride can be incorporated into

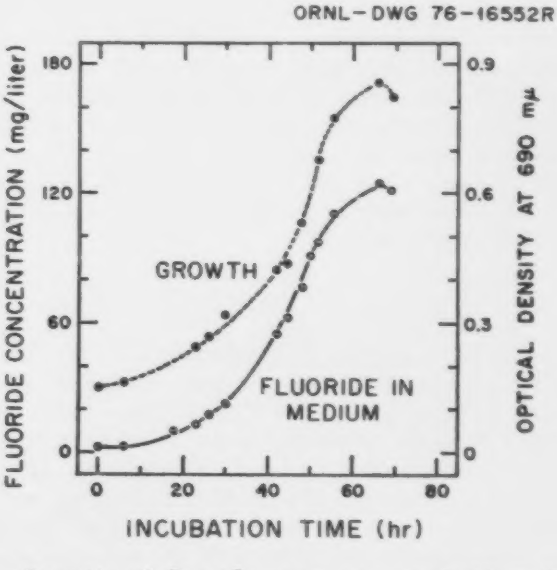

Figure 3.1. Growth of *Pseudomonas* sp. on 0.1% *p*-fluorophenylacetic acid. Source: Adapted from Harper and Blakley, 1971, Figure 1, p. 636. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Volume 17.

microbial protein via metabolism of fluoridated amino acids (Browne, Kenyon, and Hegeman, 1970; Dunn and Leach, 1967; Pine, 1978; Rennert and Anker, 1963). For example, a leucine-requiring mutant of *E. coli* (strain B 615 F) substituted 5',5',5'-trifluoroleucine for leucine in proteins (Rennert and Anker, 1963). Similarly, a cell-free *E. coli* (strain Crookes) system incorporated *p*-fluorophenylalanine into protein (Dunn and Leach, 1967), and an *E. coli* mutant strain lacking tryptophanase and tryptophan synthetase incorporated DL-tryptophan substituted with fluorine in either the 4, 5, or 6 position (Browne, Kenyon, and Hegeman, 1970).

5-Fluorouracil (FU) has been used extensively in the study of nucleic acid metabolism. Mandel (1969) reviewed the incorporation of FU into RNA and its molecular consequences. Table 3.1 shows the percent replacement

TABLE 3.1. INCORPORATION OF 5-FLUOROURACIL INTO RNA OF VARIOUS MICROBIAL SPECIES

Species	Uracil replaced (%)	Comment[b]
	Bacteria	
Escherichia coli B	70	Partially reversed by uracil
Escherichia coli, uracil auxotroph	25	Essentially unchanged by addition of uracil; 5-fluorouracil partially replaced uracil requirement
Bacillus subtilis	40-60	
Bacillus cereus	15	Reversed by uracil; enhanced by deoxythymidine
Staphylococcus aureus	5	Tested only in presence of uracil
Leuconostoc cerevisiae	*a*	Reversed by uracil
	Fungi	
Saccharomyces carlsbergensis	70	Reversed by uracil
Candida utilis	10	Reversed by uracil; incorporation of 5-fluorouracil, equal to that of uracil
Trichoderma viride	*b*	
	Virus	
Tobacco mosaic virus	56	Reversed by uridine
Polio virus	36	Reversed by uridine
Phage MS2	80	
Phage R 17	28	Reversed by uracil
Phage f2	*c*	

[a] 6.9 μg 5-fluorouracil per milligram dry weight.
[b] Tritiated 5-fluorouracil incorporated into RNA.
[c] ^{14}C-labeled 5-fluorouracil incorporated into phage RNA.

Source: Adapted from Mandel, 1969, Table 1, p. 85. Reprinted by permission of the publisher.

of uracil by FU in several bacteria, fungi, and viruses. Kaiser (1969) reported that *E. coli* B grown in the presence of FU-RNA contained a mixture of normal and FU-containing transfer ribonucleic acids (FU-*t*RNA). Up to 65% to 70% replacement of uracil residues by FU was detected in the *t*RNA. Similarly, Viriyanondha and Baxter (1970) grew *Staphylococcus aureus* ATCC 6538P in a complex medium with 300 μg per milliliter of fluoroacetate. Approximately 50% of the acetate in *N*-acylamino sugar-containing uridine nucleotides, excreted in response to penicillin, was substituted with fluoride.

Jenkins, Edgar, and Ferguson (1969) reported that plaque bacteria grown on fluoride-rich media contained high levels (up to 134 ppm) of fluoride (Table 3.2). (Data are for a *Streptococcus* strain; similar results were obtained for another *Streptococcus* and an organism tentatively identified as a *Staphylococcus*.) Dental plaque, which is mainly amassed bacteria, may contain as much as 180 ppm fluoride (Hardwick and Leach, 1963).

TABLE 3.2. FLUORIDE CONCENTRATION IN A *STREPTOCOCCUS* ISOLATED FROM HUMAN PLAQUE AND GROWN ON MEDIA CONTAINING A RANGE OF FLUORIDE WITH FINAL pH REACHED AFTER INCUBATION WITH SUCROSE FOR 18 hr[a]

Fluoride added to media (ppm)	Fluoride in suspension (μg/ml)	Nitrogen in suspension (mg/ml)	Estimated fluoride in washed bacteria (ppm)	pH after incubation
0	0.8	0.46	27	4.72
2	2.0	0.51	62	5.00
5	4.5	0.58	124	5.16
10	5.0	0.59	134	5.43

[a] These figures on fluoride concentration were obtained before the advent of the fluoride electrode and are now thought to be too high; however, later work has confirmed that bacteria take up fluoride and are inhibited as a result.

Source: Adapted from Jenkins, Edgar, and Ferguson, 1969, Table 4, p. 112. Reprinted by permission of the publisher.

High levels of fluoride can also be accumulated by certain species of lichens. Lichen thalli accumulated fluoride from exposure to ambient fluoride at a level of 4 μg of fluoride per cubic meter of air (Nash, 1971). This ability to accumulate fluoride was thought to be a function of relative humidity as indicated by the data in Table 3.3. These data show that an increase in fluoride levels in two species, *Cladonia cristatella* and *Parmelia caperata*, occurred with an increase in relative humidity. Concentrations of fluoride in *Cladonia* and *Parmelia* species, transplanted near a chemical factory known to be a source of ambient fluoride, ranged

TABLE 3.3. ACCUMULATION OF FLUORIDE BY LICHENS EXPOSED TO FOUR DAYS OF UNIFORM AMBIENT FLUORIDE LEVELS (5 μg OF FLUORIDE PER CUBIC METER) BUT UNDER VARYING RELATIVE HUMIDITY REGIMES (ppm dry weight)

Species	Accumulation at relative humidity of		
	40%	63%	87%
Cladonia cristatella	10	32	82
Parmelia caperata	11	26	89

Source: Adapted from Nash, 1971, Table 3, p. 105. Reprinted by permission of the publisher.

from 100 to 200 μg of fluoride per gram dry weight (100 to 200 ppm), compared with 8 to 28 ppm fluoride in controls (Table 3.4). Laboratory results also revealed that lichens accumulated fluoride: lichens exposed to 4 μg of fluoride per cubic meter of air accumulated 84 to 115 ppm fluoride after a nine-day exposure period, as compared with 14 to 25 ppm fluoride accumulated in lichens in control chambers. Fluoride concentrations noted in several other lichens are given in Table 3.5.

TABLE 3.4. FLUORIDE CONCENTRATIONS OF TRANSPLANTED LICHENS[a] (ppm dry weight)

Exposure time	Species	Concentration at distance from factory of	
		100 m	6000 m
One-month exposure	*Cladonia cristatella*		
June		138[b]	23
July		164[b]	28
August		180[b]	24
September		220[b]	20
October		100[b]	8
Three-month exposure	*Cladonia polycarpoides*	>220[b]	18
(July to September)	*Cladonia cristatella*	>220[b]	21
	Parmelia plittii	174[b]	22

[a] Data are means of two subsamples. Values that exceeded the calibration scale are indicated by >.

[b] Significantly different from the control at the 1% level.

Source: Adapted from Nash, 1971, Table 1, p. 105. Reprinted by permission of the publisher.

TABLE 3.5. FLUORIDE CONCENTRATIONS IN LICHENS

Species	Sample description	Distance from smelter (km)	Fluoride content (ppm)
Dermatocarpon miniatum	Saxicolous, collected near aluminum smelter		199
Peltigera rufescens	Terricolous, collected near aluminum smelter		184
Parmelia saxatilis	Saxicolous, collected downwind of aluminum smelter	1.6	47
		2.1	18
		3	17
		7	19
		11	14
Usnea subfloridana	Lignicolous, collected downwind of aluminum smelter	1.6	20
		2.1	7
		3	2
		7	2
		12	<1
Ramalina fraxinea	Trees, Invergordon, Scotland, 1969[a]		0
Usnea subfloridana	Trees, Invergordon, Scotland, 1969[a]		0
Parmelia sulcata	Corticolous, field transplant, E and NE of smelter, four months	1	990
		2	750
		4	570
		8	475
		15	190
		40	70
	Corticolous, field transplant, E and NE of smelter, 12 months	1	900
		2	700
		4	516
		8	500
		15	134

[a] Aluminum smelter began operation in spring 1971.

Source: Adapted from Gilbert, 1973, Table 2, p. 179. Data collected from several sources. Reprinted by permission of the publisher.

Although fluoride can be accumulated by some microorganisms, experimental evidence shows that in some instances the fluoride-cell bond is weak and that fluoride accumulation can be reversed. Comeau and LeBlanc (1972) found that three weeks after fumigating *Hypogymnia physodes* with 65 ppb fluoride for 2 hr, 36% and 47% of the accumulated fluoride was lost. No mechanism for the loss was postulated. Birkeland and Rölla (1972) found that *Streptococcus* strains OMZ 52-3, GS 5, and ATCC 10558 had negligible or no affinity for fluoride, either when standing in phosphate buffer (10 μM sodium fluoride) or grown in 5% sucrose broth containing 10 μM fluoride. This lack of bacterial affinity suggests that if fluoride is bound within oral bacteria, it is probably associated with a low-molecular-weight compound. Accumulation of fluoride in a beer culture after successive fermentations was studied by Klopper and Jongeling-Eijndhoven (1971). Concentrations up to 29.89 ppm fluoride occurred in *Saccharomyces carlsbergensis* cells after six successive fermentations when 10 ppm fluoride was added to the wort (Table 3.6). However, washing the cells removed

TABLE 3.6. FLUORIDE CONCENTRATIONS IN WASHED AND UNWASHED YEAST AFTER SUCCESSIVE FERMENTATION SERIES

Yeast preparation	Series number	Fluoride content (ppm dry weight) for added fluoride concentration of				
		0	1	2	5	10
Unwashed	1					
	2	0.15	1.41	1.51	7.40	12.18
	3	0.11	2.23	3.72	7.83	24.67
	4	0.22	3.01	4.82	8.41	19.79
	5	2.14	2.92	5.59	10.47	16.14
	6	0.76	3.89	7.26	20.53	29.89
Washed	1					
	2	0.06	0.05	0.08	0.35	0.12
	3	0.02	0.08	0.14	0.19	0.19
	4	0.00	0.42	0.43	0.66	0.43
	5	0.04	0.05	0.11	0.13	0.12
	6	0.10	0.06	0.37		0.19

Source: Adapted from Klopper and Jongeling-Eijndhoven, 1971, Tables II and III, p. 260. Reprinted by permission of the publisher.

most of the fluoride, suggesting that the fluoride was loosely bound to the cells. Kashket and Rodriguez (1976) found that *Streptococcus sanguis* could concentrate fluoride from dilute solutions and that part of the accumulated fluoride could be readily removed by simple washing of the cells; however, part of it was tightly bound. Because uptake was not inhibited by iodoacetamide or lack of glucose, they concluded that it was a passive process.

It is becoming clear that a large part of fluoride uptake by microorganisms can be related to the weak-acid character of hydrogen fluoride. For example, Whitford et al. (1977) concluded from their study of fluoride uptake by *Streptococcus mutans* strain 6715 that "fluoride uptake occurs by the diffusion of hydrogen fluoride and subsequent trapping of ionic fluoride." It is well known that bacteria maintain a difference in pH across the cell membrane during growth and metabolism. This difference results from: (1) the action of a membrane ATPase, which moves protons out of the cell; and (2) the proton barrier function of the membrane. Therefore, when a compound such as sodium fluoride is added to a bacterial cell suspension, it will dissociate, and depending on the pH, some of the fluoride will be in the form of hydrogen fluoride, which passes readily across the membrane. Since the cell interior is alkaline, the hydrogen fluoride will dissociate. The F^- ion cannot pass back across the membrane, therefore it is trapped within the cell. This mechanism for fluoride uptake is probably the major basis for the common finding that fluoride is more potent at lower pH values. Eisenberg et al. (1978) found that fluoride can act as a transmembrane-proton conductor to discharge the pH difference across the cell membranes of streptococci, thereby rendering them more acid sensitive.

3.2.2 Biotransformation

Many oxidation products of fluorocarbohydrates have been identified from studies of bacterial carbohydrate metabolism. Several examples of fluorocarbohydrate biotransformation by *Pseudomonas* bacteria are cited.

1. The following fluorinated intermediates were detected in the medium of a *Pseudomonas* species using *p*-fluorophenylacetic acid as its sole carbon source: (a) D(+)-monofluorosuccinic acid, (b) *trans*-3-fluoro-3-hexenedioic acid, (c) (-)-4-carboxymethyl-4-fluorobutanolide, (d) 4-fluoro-2-hydroxyphenylacetic acid, and (e) 4-fluoro-3-hydroxyphenylacetic acid (Harper and Blakley, 1971). The structures of these compounds are shown in Figure 3.2.

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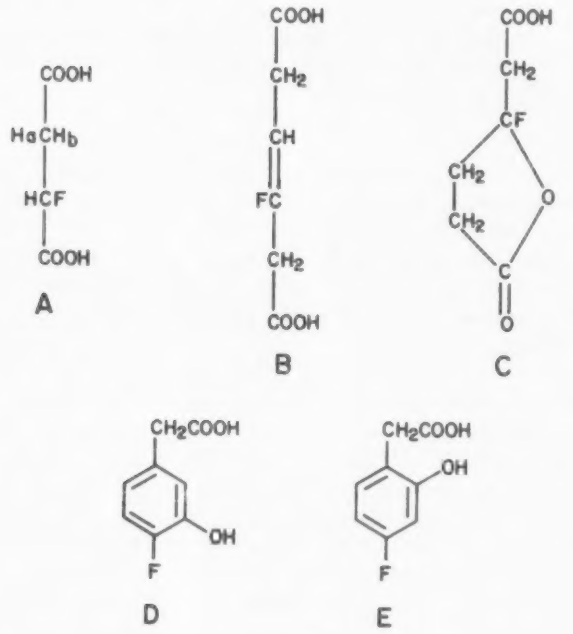
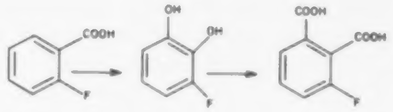


A — D(+)-monofluorosuccinic acid
B — *trans*-3-fluoro-3-hexenedioic acid
C — (—)-4-carboxymethyl-4-fluorobutanolide
D — 4-fluoro-3-hydroxyphenylacetic acid
E — 4-fluoro-2-hydroxyphenylacetic acid

Figure 3.2. Structures of metabolites isolated from medium after incubation of *Pseudomonas* sp. with *p*-fluorophenylacetic acid. Source: Adapted from Harper and Blakley, 1971, Figure 3, p. 638. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Volume 17.

2. White and Taylor (1970) reported that resting whole-cell suspensions of *Pseudomonas fluorescens* would metabolize 3-deoxy-3-fluoro-D-glucose (3FG) to 3-deoxy-3-fluoro-D-gluconic acid (3FGA). A further oxidation product was obtained and tentatively assigned the structure of 3-deoxy-3-fluoro-2-keto-D-gluconic acid (3F2KGA). Taylor, White, and Eisenthal (1972) found that *P. fluorescens* cell-free extracts immediately oxidized 3FG to 3F2KGA. Using partially purified enzyme preparations of *P. fluorescens* cell-free extracts, Taylor, Hill, and Eisenthal (1975) found that 3FG and 3FGA were substrates for glucose oxidase and gluconate dehydrogenase. Thus it seems that the same enzymes that oxidize glucose and gluconic acid also oxidize 3FG and 3FGA.

3. 3-Fluorocatechol, 2-fluoromuconic acid, and fluoride ion were identified in the growth medium of a *Pseudomonas* species which was supplied with 2-fluorobenzoic acid as its sole carbon source (Goldman, Milne, and Pignataro, 1967). The presence of the first two oxidation products suggests the following metabolic pathway:



4. A *Pseudomonas* species isolated from mud was found to selectively degrade the enantiomer of fluorocitrate that inhibits the tricarboxylic acid-cycle enzyme aconitase (Kirk and Goldman, 1970). Figure 3.3 shows the course of fluoride release due to the bacterial degradation of fluorocitrate. The concentrations of fluoride (2.1 m*M* and 2.4 m*M* in two different experiments) represent approximately half the amount of racemic *erythro*-fluorocitric acid that was added to the medium, suggesting the selective degradation of one isomer.

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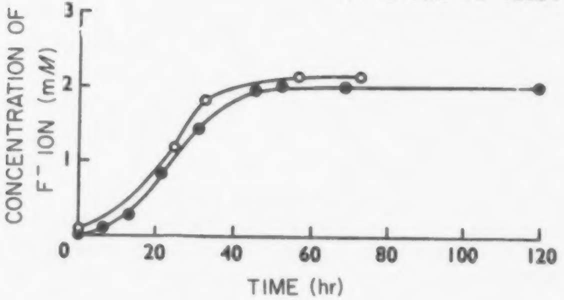


Figure 3.3. Release of F^- ion by a *Pseudomonas* sp. from racemic *erythro*-fluorocitric acid in two experiments. Source: Adapted from Kirk and Goldman, 1970, Figure 1, p. 410. Reprinted by permission of the publisher.

5. Washed cells of the actinomycete *Nocardia erythropolis* can oxidize 2-fluoro-4-nitrobenzoate to fluoroacetate, which is enzymatically converted to fluorocitrate (Cain, Tranter, and Darrah, 1968).

Although present data make it impossible to assess the relative toxicities of these fluorinated carbohydrates and ionic fluorides, organisms such as the *Pseudomonas* species discussed in example 4, which can degrade an inhibitor of aconitase, may play a role in detoxifying fluorine pollutants in the environment. For additional information on microbial biotransformation of fluorocarbohydrates see Taylor (1972) and Goldman (1972).

3.3 EFFECTS

Toxic and metabolic effects of fluoride compounds have been observed in bacteria, yeast, fungi, protozoa, algae, and viruses. Toxic effects reported include developmental and morphological alterations, growth inhibition, and reduction in infectivity. Metabolic effects include inhibition of energy-transferring mechanisms such as respiration, photosynthesis, and carbohydrate catabolism; inhibition of substrate uptake and transport; inhibition of many enzymes; and interference with protein synthesis and nucleic acid metabolism. Most of the data presented are from studies on lichen sensitivity to pollutants, the cariostatic activity of fluoride, the effects of aerosols, and the use of fluoride as an enzymatic and metabolic probe.

3.3.1 Toxic Effects

Since many lichens are sensitive to airborne fluorides, they are frequently used as indicators of fluoride air pollution (Gilbert, 1973; Hawksworth, 1971). Injury to lichen flora in the vicinity of aluminum smelters and chemical factories has been reported by several authors (LeBlanc, Comeau, and Rao, 1971; LeBlanc and De Sloover, 1970; LeBlanc, Rao, and Comeau, 1972; Pišút and Lisická-Jelínková, 1974). Severity of injury depends on distance from the fluoride source, topography, and wind direction. Toxic symptoms resulting from fluoride pollution include: chlorosis; necrosis; curled margins; white, pink, or gray colors; disintegration of the thallus; microscopically observed plasmolysis; and loss of color in the algae cells (Comeau and LeBlanc, 1972; Gilbert, 1973; Nash, 1971). In a laboratory fumigation experiment, Comeau and LeBlanc (1972) exposed *Hypogymnia physodes* to concentrations of hydrogen fluoride ranging from 13 to 130 ppb. Fumigation with 13 ppb for 8 hr caused no observable symptoms. However, 36-, 72-, and 108-hr exposures caused chlorosis and curling of the margins; so did a 12-hr exposure to 65 ppb. The authors noted that fluoride was accumulated even when no symptoms could be observed. Nash (1971) reported that lichens fumigated with fluoride invariably became chlorotic upon fluoride accumulation, and that a critical level of F^- in the thallus was 30 to 80 ppm. He concluded that the algal component of the lichen was more sensitive to hydrogen fluoride than the fungal component, since the morphological form of the lichen was maintained for several weeks after the appearance of total chlorosis.

Some toxic effects of fluorine compounds on protozoa have been observed; however, exact mechanisms of action are not known. The amino acid analog DL-*p*-fluorophenylalanine (*p*-FPhe) inhibits division in cells selected from exponentially growing populations of *Tetrahymena pyriformis* (Rasmussen and Zeuthen, 1962) and *Paramecium aurelia* (Rasmussen, 1967). In *P. aurelia*, 16 m*M* *p*-FPhe inhibited cell division when applied up to 4.5 hr after the previous cell division. After that time (the sensitivity transition point), *p*-FPhe did not block cell division. For both organisms, this transition point occurred about 1 hr before completion of the cell cycle (i.e., cytokinesis) and roughly coincided with the termination of macronuclear DNA synthesis. In a study of various chemicals associated with operation and maintenance of nuclear power plants and cooling tower structures, Becker and Thatcher (1973) reported that although fluoride ions apparently have direct toxic properties toward aquatic life, concentrations of 1.5 ppm fluoride did not appear to be harmful to aquatic organisms under most conditions. The toxic threshold of *Microregma heterostoma* occurred after a 28-hr incubation period at 27°C with 226 ppm fluoride (Bringmann and Kuhn, 1959, as cited in Becker and Thatcher, 1973).

Bean plants (*Phaseolus vulgaris* L.) showed increased numbers of lesions with increasing foliar fluoride concentrations (up to 500 ppm) after inoculation with tobacco mosaic virus (TMV) (Treshow, Dean, and Harner, 1967). Above 500 ppm fluoride the number of lesions decreased. Although fluoride did increase the virulence of TMV in this controlled situation, the authors suggested that, due to many environmental factors (e.g., light, temperature, and moisture) this increased virulence may go undetected in the field.

Effects of *p*-fluorophenylalanine on nitrous acid mutagenesis in bacteriophage T_4 *r* mutants were studied by Johnson (1975*b*). The frequency of nitrous acid-induced forward *r* mutations was approximately doubled when the phage-infected *E. coli* B cells were held in the presence of *p*-FPhe. However, spontaneous forward mutation frequency and the nitrous acid specificity were not affected by *p*-FPhe. The frequencies of spontaneous and nitrous acid-induced reversion of *rII* transition mutants were unaffected by *p*-FPhe when the phage was plated on the restrictive host after mutagenesis. However, compared with the control phage, the induced reversion frequency was approximately tripled when the mutagenized phage first underwent a cycle of replication in the presence of *p*-FPhe. In contrast, Johnston (1975*a*) reported that *p*-FPhe depressed 5-bromouracil–induced frequencies in both forward and reverse mutation. Davies and Parry (1978) found that *p*-fluorophenylalanine at growth-inhibitory concentrations increased the frequency of mitotic gene conversion in haploid yeast cultures and enhanced the mutagenicity of nitrous oxide, mitomycin C, ethylmethane-sulphonate, and uv light. They suggested that *p*-fluorophenylalanine became incorporated into the enzymes involved in DNA replication with the net result of lower fidelity of replication and increased errors in base incorporation.

Haploidization in yeast can be induced by *p*-FPhe. Gutz (1966) reported that *p*-FPhe treatment resulted in haploidization of diploid *Schizosaccharomyces pombe* strains when plated on yeast extract – glucose agar gradient plates (0.06% to 0.1% *p*-FPhe). DL-*p*-Fluorophenylalanine also induced haploidization in *Aspergillus niger* diploids (Lhoas, 1961). Conidia were plated onto standard complete medium containing 0.01% *p*-FPhe. The viable counts were the same as controls; however, the growth was slower (reduced colony diameters), and sporulation was poor.

Fluoride can interfere with fungal sporulation, germination, and growth. Lukens and Horsfall (1973), studying effects of several respiratory inhibitors on the sporulation process of *Alternaria solani*, found that 5.96 m*M* sodium fluoride reduced stalk formation by 50%. The effective concentration for 50% inhibition of conidial formation (ED_{50}) was greater than 20 m*M* and for stalk collapse, greater than 100 m*M*. Inhibition of spore germination in the dermatophyte *Microsporum gypseum* resulted from the addition of 0.1 m*M* phenylmethylsulfonyl fluoride to the germination medium (Leighton and Stock, 1970). No effect on growth occurred if phenylmethylsulfonyl fluoride was added after the emergence of the germ tube. The compound was found to specifically inhibit the release of free amino nitrogen by the macroconidia. Sodium fluoride at a concentration of 50 m*M* and administered to spores which had been cultured for 4 hr at 30°C, affected conidia production of *Neurospora crassa* strain 853 A (Figure 3.4) (Timberlake and Turian, 1975). The effects were time-dependent. Conidiation increased with length of treatment up to 2 hr, stabilized from 2 hr

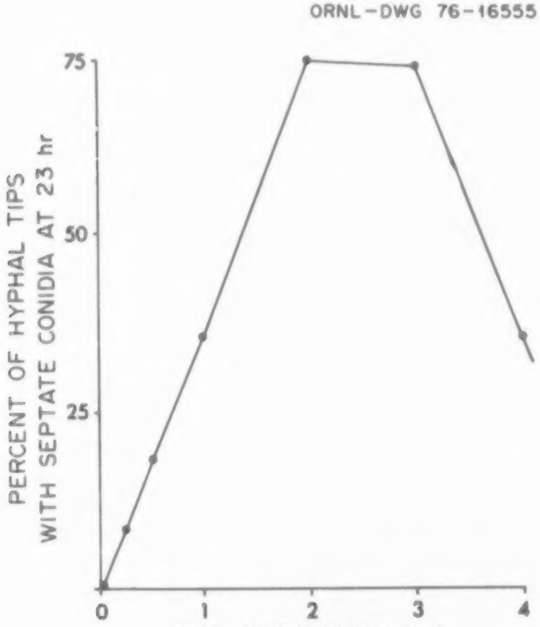


Figure 3.4. The effect of the duration of 50 m*M* NaF treatment on production of conidia; initial treatment was to 4-hr-old *Neurospora crassa* cultures. Source: Adapted from Timberlake and Turian, 1975, Figure 1, p. 152. Reprinted by permission of the publisher.

to 3 hr, and then decreased after 3 hr. The growth of the treated cultures was as rapid as that of the untreated, until the 3-hr treatment period. The inhibition could be reversed by washing or by the addition of magnesium chloride. Treshow (1965) found varying effects on fungal growth and sporulation from concentrations of 0.1 to 50 m*M* sodium fluoride. *Verticillium albo-atrum* growth was completely inhibited by 50 m*M* fluoride, and *Colletotrichum lindemuthianum* was the only fungus that grew at all in this concentration. Table 3.7 shows other reported effects (Treshow, 1965). Leslie and Parbery (1972) found that *Verticillium lecanii* grew readily on 211 m*M* fluoride, a tolerable concentration four times that reported by Treshow (1965) for *V. albo-atrum*. No differences in the morphology of *V. lecanii*, resulting from growth on fluoride-containing medium, were noted by Leslie and Parbery. However, spore length increased with increasing fluoride concentrations. Fluoroamino acids such as DL-*p*-fluorophenylalanine (*p*-FPhe) can also inhibit bacterial sporulation (Hardwick and Foster, 1952), even at concentrations that have little effect on growth (Marquis, 1970).

TABLE 3.7. EFFECTS OF SODIUM FLUORIDE ON GROWTH AND SPORULATION OF PATHOGENIC FUNGI

Species	NaF concentration (m*M*)	Effect on growth rate	Effect on sporulation
Alternaria oleraceae	≥5	Significantly suppressed at 21°C	No effect at ≤1 m*M*
	25, 50	Complete inhibition	
Botrytis cinerea	≥5	Significantly suppressed	
	0.1, 0.5, 1	Significant stimulation	
	5		Reduced sporulation; only a few conidiospores matured
	10		Fewer conidiospores matured than at 5 m*M*
Colletotrichum lindemuthianum	1	Stimulated at 24°C	No effect
	50	Suppressed at 21°C and 24°C	No effect
Cytospora rubescens	1	Significantly suppressed at 24°C	
	5	Significantly suppressed at 21°C	
	≥10	Complete inhibition	
Helminthosporium sativum	<25	No effect	No effect until complete growth inhibition
Pythium debaryanum	0.5	Significantly inhibited at 27°C	
	10	Significantly inhibited at 24°C	
	25	Significantly inhibited at 21°C	
Verticillium albo-atrum	25	Significantly inhibited at 27°C	
	1	Stimulated at 18°C or 27°C; not stimulated at 21°C or 24°C	

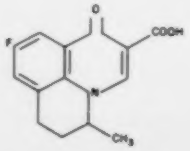
Source: Adapted from Treshow, 1965, Figure 1, p. 217. Reprinted with permission from Mycologia 57:216-221, 1965.

With the increased use of aerosol propellants, the amount of fluorocarbons in the atmosphere has increased (Section 7.3.4). It is important to know the effects of these propellants on microorganisms and also to know if these compounds might be useful as disinfectants for use in foods or for human infections. Effects of some of these fluorocarbons on microorganisms are listed in Table 3.8. Bacterial spores seem to be more resistant than vegetative cells. Some treatments have resulted in increased survival rates or only slight toxicity. Oujesky and Bhagat (1973) examined the effects of several Freons and Genetrons on both coagulase-positive and coagulase-negative strains of *S. aureus*. The most effective gas tested was dichlorofluoromethane (Genetron-21, Freon-21), which inhibited both coagulase-positive and coagulase-negative strains (Table 3.9). The coagulase-negative strains (Guinn and ATCC 6020) were more sensitive than the coagulase-positive (Giorgio) strain. There was no apparent effect on deoxyribonuclease and gelatinase production by the three strains. The higher molecular-weight ethane and methane compounds and those containing more chloride than fluoride appeared to be the most toxic. Thus, the toxicity of chloride-containing fluorocarbon propellants may be enhanced by chloride substitution for fluoride (Section 7.3.4). From their results, Prior et al. (1975) suggested that the death of *E. coli* cells, caused by dichlorodifluoromethane, involved increased permeability, leakage of cell constituents, and lysis. The fluorocarbon liquids used in experiments on liquid breathing and for formulations of artificial blood do not appear to be toxic to microorganisms and have been used to supply oxygen to pressurized cultures (Marquis, 1976).

The physiological effects on *E. coli* of incorporated amino acid analogues were described by Pine (1978). He determined a toxicity series with canavanine > azetidine-2-carboxylate > *p*-fluorophenylalanine > ethionine > norleucine or *o*-fluorophenylalanine > *m*-fluorophenylalanine or 2,5-dihydrophenylalanine > selenomethionine. Kay and Cameron (1978*a*, 1978*b*) used the fluoridated acids fluorocitrate and fluoromalate to isolate transport-negative mutants of *S. typhimurium* for citrate and dicarboxylic acids respectively.

Gallon, Ul-Haque, and Chaplin (1978) found that among the pleiotrophic effects of fluorocitrate on the blue-green bacterium *Gloeocapsa* sp. was an inhibition of nitrogen fixation, especially under aerobic conditions. Ammonium ion accumulated within the organism apparently because of the reduced synthesis of ketoglutarate needed for its assimilation. The lipid content of the organism was also altered.

A new fluoroacid, flumequine, has the following structure:



Greenwood (1978) found that flumequine was active against *E. coli* and could possibly be used in place of nalidixic acid for treatment of urinary tract infections.

TABLE 3.8. EFFECTS OF FLUOROCARBON AEROSOL PROPELLANTS ON MICROORGANISMS

Compound	Treatment	Species	Effect	Reference
Fluorocarbon-12[a] and fluorocarbon-114[b] (mixture)	40/60 propellant blend (12/114)	*Pseudomonas aeruginosa*	Growth not affected at 37°C for 48 hr	Reed and Dychdala, 1964
		Staphylococcus aureus	Growth not affected at 37°C for 48 hr	Reed and Dychdala, 1964
		Streptococcus agalactiae (microaerophile)	Growth inhibited	Reed and Dychdala, 1964
		Aspergillus niger (fungus)	Growth inhibited	Reed and Dychdala, 1964
		Paecilomyces varioti (fungus)	Growth inhibited	Reed and Dychdala, 1964
Fluorocarbon-12, fluorocarbon-142b[c]	Vapor and liquid states[d]	*Staphlococcus aureus*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Micrococcus conglomeratus*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Streptococcus cremoris*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Streptococcus lactis*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Leuconostoc citrovorum*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Leuconostoc dextranicum*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Bacillus cereus* (spores)		Prior, Fennema, and Marth, 1970
	Vapor state	*Bacillus polymyxa* (spores)		Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Escherichia coli*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Escherichia intermedia*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Salmonella typhimurium*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Pseudomonas aeruginosa*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor and liquid states	*Pseudomonas fluorescens*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Pseudomonas fragi*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Achromobacter butyri*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Flavobacterium devorans*	Growth inhibited	Prior, Fennema, and Marth, 1970

TABLE 3.8 (continued)

Compound	Treatment	Species	Effect	Reference
	Vapor and liquid states	*Saccharomyces cerevisiae*	Growth inhibited	Prior, Fennema, and Marth, 1970
	Vapor state	*Candida utilis*	Growth inhibited	Prior, Fennema, and Marth, 1970
Fluorocarbon-11[e] and fluorocarbon-12 (mixture)	Vapor mixture (60:40)	*Escherichia coli*	Rate of inactivation slower than Prior et al. (1970) results; slowly lethal – 1.0% survivors after treatment for three weeks	Stretton, Gretton, and Watson-Walker, 1971
		Bacillus subtilis var. *niger*	Spores more resistant than *E. coli* vegetative cells	Stretton, Gretton, and Watson-Walker, 1971
Genetron-21[f]	5-min gassing treatment (30 ml/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase positive strain	Decreased survival rate	Oujesky and Bhagat, 1973
	5-min gassing treatment (30 mg/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase negative strain	Decreased survival rate	Oujesky and Bhagat, 1973
Genetron-23[g]	5-min gassing treatment (30 ml/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase positive strain	Increased survival rate and coagulase production	Oujesky and Bhagat, 1973
	5-min gassing treatment (30 mg/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase negative strain	Decreased survival rate	Oujesky and Bhagat, 1973
Genetron-152A[h]	5-min gassing treatment (30 ml/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase positive strain	Increased survival rate and coagulase production	Oujesky and Bhagat, 1973
	5-min gassing treatment (30 mg/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase negative strain	Increased cell viability	Oujesky and Bhagat, 1973
Fluorocarbon-12	5-min gassing treatment (30 ml/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase positive strain	Increased survival rate and coagulase production	Oujesky and Bhagat, 1973
	5-min gassing treatment (30 mg/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase negative strain	Increased cell viability	Oujesky and Bhagat, 1973
	Liquid, 2 hr, 22°C	*Escherichia coli* H52	Inactivated young cells more rapidly than old cells; clumping; cytoplasm light and grainy – less dense and less uniform than control	Prior, Fennema, and Pate, 1975

TABLE 3.8 (continued)

Compound	Treatment	Species	Effect	Reference
	1.25 × saturation for up to 1200 min, 22°C	*Escherichia coli* ML30	Decreased viability; 50% loss of compounds absorbing at 260 nm; 32% lysis; most changes in first 300 min	Prior, Fennema, and Pate, 1975
Fluorocarbon-114	5-min gassing treatment (30 ml/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase positive strain	Enhanced coagulase production and mannitol fermentation; decreased survival rate	Oujesky and Bhagat, 1973
	5-min gassing treatment (30 mg/min); then 24 hr in gaseous atmosphere	*Staphylococcus aureus*, coagulase negative strain	Decreased survival rate	Oujesky and Bhagat, 1973
Fluorocarbon-21	0.5% to 1.5% (wt/wt)	*Saccharomyces cerevisiae*	Inactivation greatest with (1) increasing concentrations, (2) increasing volume of medium, (3) increasing temperature from 7°C to 47°C, (4) agitation, (5) young cells (8 hr) rather than old (36 hr or 10 days), and (6) cells grown without glucose; treated cells resistant to lysis by sonication	Middleton, Marth, and Fennema, 1975

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[a] Freon-12, dichlorodifluoromethane.

[b] Freon-114, 1,2-dichlorotetrafluoroethane.

[c] 1,1-Difluoro-1-chloroethane.

[d] Agitated for 48 hr at 21°C. Vapor state: vapor space saturated at 21°C, but amount insufficient to cause condensation. Liquid state (partial): vapor space saturated; at low level, few drops of condensate in medium; at high level enough condensate in medium to bind all water.

[e] Trichlorofluoromethane.

[f] Fluorocarbon-21, dichlorofluoromethane.

[g] Trifluoromethane.

[h] 1,1-Difluoroethane.

TABLE 3.9. SURVIVAL OF A COAGULASE-POSITIVE (GIORGIO) STRAIN AND TWO COAGULASE-NEGATIVE STRAINS (GUINN AND ATCC 6020) OF *STAPHYLOCOCCUS AUREUS* AS DETERMINED BY COLONY COUNTS AFTER EXPOSING THE BACTERIAL CELLS TO 5-min GASSING TREATMENT (30 ml/min) AND A SUBSEQUENT 24 hr IN THE GASEOUS ATMOSPHERE[a]

Gas used for treatment	Strain	Untreated controls (cells/ml)	Viable gas-treated cells (cells/ml)	Survivors from gas-treated cells (% ± std deviation)
		Coagulase positive		
Air, compressed		1.6×10^8	1.8×10^8	115.0 ± 14.0
Genetron-152A		1.0×10^8	1.0×10^8	106.0 ± 26.0
Genetron-23		0.8×10^8	1.4×10^8	185.0 ± 19.0
Genetron-21		1.7×10^8	0.4×10^6	0.2 ± 0.09
Freon-12		1.1×10^8	1.6×10^8	148.0 ± 5.0
Freon-114		1.2×10^8	1.0×10^8	84.3 ± 4.9
		Coagulase negative		
Air	Guinn	1.8×10^8	1.9×10^8	108.0 ± 9.0
	ATCC 6020	1.9×10^7	2.3×10^7	121.0 ± 2.8
Genetron-152A	Guinn	1.5×10^8	1.8×10^8	118.4 ± 13.6
	ATCC 6020	1.9×10^7	1.8×10^7	95.0 ± 17.3
Genetron-23	Guinn	1.3×10^8	0.9×10^8	71.6 ± 19.4
	ATCC 6020	1.2×10^7	1.0×10^7	85.0 ± 12.05
Genetron-21	Guinn	1.4×10^8	0.3×10^4	0.0024 ± 0.0016
	ATCC 6020	2.7×10^7	0	0
Freon-12	Guinn	1.0×10^8	1.3×10^8	126.0 ± 18.4
	ATCC 6020	1.9×10^7	2.4×10^7	124.5 ± 5.5
Freon-114	Guinn	1.1×10^8	0.9×10^8	80.0 ± 7.0
	ATCC 6020	1.6×10^7	0.9×10^7	55.9 ± 4.1

[a] Coagulase-positive organisms were incubated on coagulase mannitol agar for 24 hr at 37°C; coagulase-negative organisms were incubated on brain heart infusion agar for 24 hr at 37°C.

Source: Adapted from Oujesky and Bhagat, 1973, Table 3, p. 232, and Table 6, p. 234. Reprinted by permission of the publisher.

Some fluorinated bases are used as antimicrobial, and often antitumor, agents. For example, 5-trifluoromethyl-2'-deoxyuridine has been used to treat herpes virus infections. It appears (Clough, Wigdahl, and Parkhurst, 1978) that this compound is hydrolyzed to yield 5-carboxy-2'-deoxyuridine, which is the active agent that upsets DNA synthesis at the level of the de novo biosynthetic pathway for pyrimidine biosynthesis. Fluoridated compounds are also widely used as anesthetics. Among the best known ones are halothane (2-bromo-2-chloro-1,1,1 trifluoroethane) and methoxyflurane ($CHCl_3$-CF_2-OCH_3). These agents are active against microorganisms and microbial responses. Many of these responses seem to involve the loss of normal microtubular structure as shown in the following examples: loss of motility of *Tetrahymena pyriformis* (Nunn, Dixon, and Moore, 1968); immobilization and stoppage of cytoplasmic streaming in amoebae (Bruce and Christiansen, 1965); collapse of heliozoan axopods (Allison et al., 1970); inhibition of cell division of *Tetrahymena* (Kirkness and MacDonald, 1972); and inhibition of bacterial luminescence (Halsey and Smith, 1970).

A very dramatic bacterial response to growth in fluoridated media was described by Lesher, Bender, and Marquis (1977). Massive lysis occurred in cultures of *Bacillus subtilis*, *Neisseria subflava*, and a micrococcus called LYT following growth in the presence of 5 or 10 m*M* sodium fluoride. The action appeared to be the result of activation of autolytic enzymes rather than to any restriction in the supply of phosphoenolpyruvate, the production of the enolase reaction needed for the synthesis of the cell-wall structural polymer peptidoglycan. Only bacteria with active autolytic systems underwent lysis, but increased turnover of peptidoglycan occurred in other organisms during growth in fluoridated media. Therefore, in some instances, it is evident that fluoride can be bactericidal instead of only bacteriostatic.

It appears also that some fluoride preparations can actually kill bacteria and serve as deplaquing agents as well as cariostatic agents. For example, Mühlemann and Strub (1975) reported a 21.5% reduction in the sulcular plaque index due to daily rinsing by human subjects with 0.025% amine fluoride solution. Andres, Shaeffer, and Windeler (1974) found that mouth washing with 0.5% SnF_2 solution, but not 0.27% sodium fluoride solution, reduced the number of viable bacteria in saliva. Tinanoff, Brady, and Gross (1976) showed that daily mouth rinsing with SnF_2 solution (100 ppm fluoride) reduced early plaque formation, while sodium fluoride at an equivalent fluoride concentration was ineffective with once-a-day application but effective when used twice a day.

The antiplaque activity of topical fluoride preparations apparently has a number of bases. For one, it appears that fluoride can inhibit initial colonization of hydroxyapatite by oral bacteria (Rölla, 1977). In addition, the counterion for fluoride may cause more damage than the fluoride itself. Thus, Sn^{2+}, alkyl amines, and a pH of 3.2 are all very damaging to bacterial cells. However, it should be pointed out that sodium fluoride or potassium fluoride solutions can act as deplaquing agents when they are used frequently, and it seems that it must be the fluoride in those solutions that is the active agent.

There has been considerable use in caries prevention of monofluorophosphate, especially in fluoride toothpastes. However, recent studies by Pearce and Jenkins (1976, 1977) indicated that fluorophosphate was no more effective than sodium fluoride in reducing acid production in human saliva and that, in fact, it is decomposed by saliva to yield fluoride, which is probably the active inhibitory agent.

Many strains of oral bacteria can become acclimatized to fluoride. This acclimation process was studied in several oral streptococcal strains (Williams, 1964, 1967, 1968). Williams (1964) found that several strains of *Streptococcus faecalis* adapted to sodium fluoride (concentrations not reported). These fluoride-adapted cells exhibited a longer lag phase than control cells, whether in the presence or absence of fluoride. Five strains of Lancefield group D streptococci — HT25 and 680 (isolated from human dental plaque), *Streptococcus zymogenes* NCIB 8886, *Streptococcus faecalis* NCDO 580, and *Streptococcus faecalis* NCTC 370 — were "fluoride trained" by growing in 2 m*M* fluoride for four days, then for four days in 10 m*M*, 33 m*M*, and 100 m*M* fluoride successively. The adapted cultures grew more successfully in high fluoride concentrations than control strains in the absence of fluoride. If these cultures were grown for two to three days in fluoride-free medium, they lost their fluoride resistance. Using strain HT25, Williams (1968) found that both fluoride-acclimated cells and control cells were quite permeable to fluoride; however, the acclimated cells appeared to be less permeable. Perhaps the difference was due to factors such as differences in culture, pH, and growth phase. The bacteria were grown overnight in 8.5 ppm fluoride at 37°C, and the volume of cell protoplasts was estimated using a radioactive sulfate technique.

Permanent or genotypic resistance to fluoride in *S. salivarius* was studied by Hamilton (1977). Hamilton also noted that slowly metabolizing bacteria are more fluoride resistant than rapidly metabolizing bacteria. Hamilton and Ellwood (1978) found that cells of *S. mutans* strain Ingbritt, grown anaerobically in a chemostat at pH 5.5, were more fluoride resistant and had higher glycolytic capacities than cells grown at higher pH values of 6.0 or 6.5.

Frostell and Ericsson (1978) stated that bacteria readily became resistant to fluoride when grown in its presence and that dental-plaque bacteria in areas with fluoridated water acquired fluoride tolerance. It should be noted that fluoride intake in areas of the United States without water fluoridation may be nearly as great as that in fluoridated areas because of consumption of foods and beverages processed with fluoridated water. Rosen, Frea, and Hsu (1978) indicated that fluoride-resistant variants of *S. mutans* were less cariogenic than parent strains, and that the carious process produced by the variants could be suppressed by fluoride. They concluded that their findings favored a mechanism for the anticaries activity of fluoride that does not involve direct inhibition of bacterial metabolism. However, some caution is needed because many fluoride-resistant mutants, for example those studied by Hamilton (1977), are resistant at pH values near neutrality but are still sensitive at lower pH values. Clearly, dental-plaque bacteria have had to adapt to the presence of millimolar levels of fluoride.

Herbison and Handelman (1975) tested the effects of fluoride on five antigenic strains (AHT, BHT, GS-5, LM-7, and SLIR; antigenic types *a*, *b*, *c*, *d*, and *E* respectively) of *Streptococcus mutans* in vitro. Fluoride (0.15, 1.5, and 15 ppm) reduced hydroxyapatite solubility in three strains. In combination with strontium (1.7, 17.0, and 170 ppm), solubilization was reduced even further. Fluoride at 1.5 ppm reduced acid production in the five strains. This reduction was negated by the addition of 17 ppm strontium. No significant effect was observed in four strains grown on agar plates that were treated with fluoride at concentrations of 0.15, 1.5, and 15 ppm.

The cariogenic K-1 strain of *S. mutans* has been the subject of much study on the anticaries effect of fluoride (Luoma, 1972*a*, 1972*b*, 1973; Luoma, Ranta, and Turtola, 1971). The addition of fluoride causes leakage of potassium and phosphorus from both fermenting and nonfermenting cells and reduces acid production. The current opinion by many researchers is that fluoride does not directly inhibit the phosphotransferase system for sugar uptake. Schachtele and Mayo (1973) found no such inhibition. However, fluoride can indirectly inhibit the system by inhibiting the enolase reaction. Also, Marquis (1977) found that fluoride could inhibit streptococcal membrane ATPase. This inhibition could reduce potassium uptake by the cells.

At pH values of 5.2 and 5.8, 50 ppm fluoride caused leakage of cellular potassium in fermenting systems (Luoma, 1972*a*). At higher pH levels (6.8 and 8.4), 100 ppm fluoride slightly reduced cellular potassium leakage. Twenty-five ppm fluoride, alone or in combination with 6% ethanol, reduced the cellular potassium and phosphorus levels in nonfermenting cells. The concentration of phosphorus was less affected. Chlorhexidine, a compound used effectively to remove dental plaque (Gjermo and Rölla, 1971, as cited in Luoma, 1972*b*), had previously been found to cause leakage of cellular phosphorus in micrococci (Rye and Wiseman, 1964, as cited in Luoma, 1972*a*). Luoma (1972*b*) tested the action of fluoride on strain K-1 of *S. mutans* at concentrations of 25, 50, 75, and 100 ppm alone or in combination with chlorhexidine (12.5 to 50.0 ppm). He found reduced cellular potassium and phosphorus concentrations in fermenting cells. Sodium levels increased in cells incubated without sucrose at 50 ppm fluoride, 12.5 ppm chlorhexidine, or a combination of both. If the incubation mixture contained sucrose, cells treated with 50 ppm fluoride or 50 ppm fluoride plus 12.5 ppm chlorhexidine had about the same sodium concentration as untreated systems. A pretreatment with 50 ppm fluoride had little effect on acid production by strain K-1; however, 50 ppm fluoride plus 50 ppm chlorhexidine completely inhibited acid production. Further testing (Luoma, 1973) showed that fluoride in combination with chlorhexidine and propanol produced the greatest reductions in potassium and phosphorus concentrations in the cells and the greatest reduction in acid production. The results of Luoma's research are interesting, but they have not been fully interpreted.

Loesche et al. (1975) found that acidulated phosphate fluoride gel may be an effective oral antimicrobial agent. They compared the percentage of *S. mutans* and *S. sanguis* present in dental plaque samples before and after treatment with acidulated phosphate fluoride. Prior to treatment, *S. mutans* comprised about 9% of the colony-forming units in the occlusal plaque and 1% in the approximal plaque. *S. sanguis* comprised about 5% of the colony-forming units in the approximal plaque and 8% in the occlusal plaque. In the test, 10 ml of gel containing 1.23% fluoride ion in 0.1 *M* phosphoric acid solution at pH 3.2 was applied 8 to 10 times daily for 8 to 10 days to 44 boys, 14 to 16 years old. The acidulated phosphate fluoride caused reductions of 45% to 75% of *S. mutans* in occlusal samples but had no effect in approximal samples. No effect was observed on *S. sanguis* populations.

3.3.2 Metabolic Effects

This discussion on microbial metabolic effects caused by fluoride compounds deals with four topics: (1) energy transfer, (2) carbohydrate metabolism, (3) protein synthesis and nucleic acid metabolism, and (4) enzymatic activity.

3.3.2.1 Energy Transfer -- Inorganic fluoride can interfere with microbial energy-transfer processes. Examples are inhibition of respiration, photosynthesis, and carbohydrate metabolism (Section 3.3.2.2). Effects of sodium fluoride on respiration of the aquatic fungi *Allomyces javanicus* and *Brevilegnia unisperma* var. *delica* in the presence and absence of glucose are shown in Table 3.10 (Thakur and Dayal, 1971); inhibition was greatest in the presence of glucose. A concentration of 10 m*M* fluoride caused 95% inhibition of *A. javanicus* respiration. Studies involving the green algae *Chlorella vulgaris* and *Chlorella pyrenoidosa* showed that endogenous respiration rates were not decreased by the individual addition of 40 m*M* sodium fluoride or 8 m*M* copper sulfate. However, if both agents were added simultaneously, oxygen uptake was almost completely inhibited (Hassall, 1967; Sargent and Taylor, 1972). Sodium fluoride (50 m*M*) treatment of dormant conidia of *N. crassa* induced premature conidia germination and also decreased the rate of respiration (Figure 3.5) (Timberlake and Turian, 1975).

TABLE 3.10. EFFECTS OF FLUORIDE ON RESPIRATION OF THE AQUATIC FUNGI *ALLOMYCES JAVANICUS* AND *BREVILEGNIA UNISPERMA* VAR. *DELICA*

Treatment	*Allomyces javanicus*		*Brevilegnia unisperma*	
	Molecular oxygen as percent of control	Percent inhibition	Molecular oxygen as percent of control	Percent inhibition
Control	175	0	110	0
In absence of glucose				
0.01 *M* fluoride	127	48	80	30
0.005 *M* fluoride	148	27	93	17
In presence of glucose				
0.01 *M* fluoride	80	95	66	44
0.005 *M* fluoride	137	38	83	27

Source: Adapted from Thakur and Dayal, 1971, Table 2, p. 467. Reprinted by permission of the publisher.

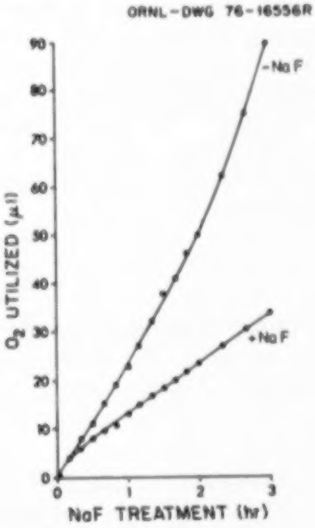

Figure 3.5. Oxygen uptake during NaF treatment of *Neurospora crassa*. Source: Adapted from Timberlake and Turian, 1975, Figure 5, p. 153. Reprinted by permission of the publisher.

Fluoride has been used as an inhibitor of photosynthesis in the green alga *Chlorella* (Warburg, 1962) and of photosynthesis and the quinone Hill reaction in the blue-green bacteria *Plectonema boryanum* and *Anabaena variabilis* (Vennesland and Turkington, 1966*a*, 1966*b*). Potassium fluoride at a concentration of 20 m*M* in the absence of carbon dioxide and at pH 5.8 almost completely inhibited the quinone Hill reaction in washed suspensions of *P. boryanum* and *A. variabilis*. Fluoride inhibition increased with decreasing pH. Figure 3.6 illustrates the effect of fluoride at pH 5.2 on photosynthesis and on the Hill reaction in *P. boryanum* cell suspensions. Photosynthesis was more sensitive to fluoride than was the Hill reaction.

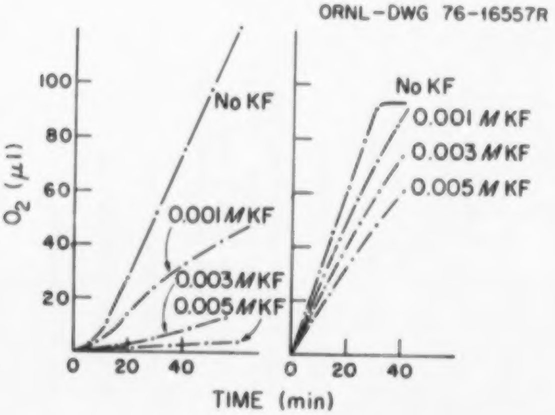

Figure 3.6. Effect of fluoride at pH 5.2 on photosynthesis (left) and on the Hill reaction (right) in *Plectonema boryanum* cell suspensions. Source: Adapted from Vennesland and Turkington, 1966*a*, Figure 2, p. 154. Reprinted by permission of the publisher.

3.3.2.2 Carbohydrate Metabolism — Fluoride influences several aspects of carbohydrate metabolism, including sugar uptake, acid production, and enzyme activities. Many of these studies have involved cariogenic bacteria and attempts at elucidating the mode of action of fluoride in reduction of caries. Information indicates that the effect of fluoride on sugar metabolism involves bacterial membranes (Luoma and Tuompo, 1975; Schachtele and Mayo, 1973). Table 3.11 lists some effects of fluorides on several aspects of microbial carbohydrate metabolism. The concentrations tested ranged from 0.01 to 47.5 m*M* sodium fluoride. At low fluoride concentrations, stimulation of phosphorylase and carbon dioxide formation in salivary organisms and of glucose fermentation by *E. coli* are reported (Table 3.11).

Weiss et al. (1965) found that fluoride at concentrations as low as 1 to 10 ppm affected the rate of synthesis of iodophilic polysaccharides from glucose, maltose, and sucrose by *Streptococcus mitis*; fluoride inhibition was reversed with time. Figure 3.7 shows the rapid effect of fluoride at

TABLE 3.11. EFFECTS OF FLUORIDE (NaF) ON MICROBIAL CARBOHYDRATE METABOLISM

Organism	Concentration (mM)	Effect	Reference
Escherichia coli	0.01	Activation of glucose fermentation	Venkateswarlu, 1970
Yeast	0.05	Activation of fermentation	Venkateswarlu, 1970
Aspergillus niger	0.05	Inhibition of citric acid production	Venkateswarlu, 1970
Streptococcus and *Lactobacillus* sp.	0.01	Inhibition of acid production	Venkateswarlu, 1970
Yeast	0.1	Inhibition of pyrophosphatase	Venkateswarlu, 1970
Streptococcus salivarius	2.4	Inhibition of glucose uptake; reduction in cellular glucose-6-P; inhibition of intracellular enolase activity	Kanapka and Hamilton, 1971
	0.06	Decrease in cellular glucose-6-P in cells metabolizing glucose if added before or after substrate	Kanapka, Khandelwal, and Hamilton, 1971
	1.2	Stimulation of phosphorylase activity	Kanapka, Khandelwal, and Hamilton, 1971
	2.4	Stimulation of phosphorylase activity; immediate decrease in cellular glucose-6-P and ATP in cells actively degrading glucose; complete inhibition of glucose uptake and glycogen synthesis	Kanapka, Khandelwal, and Hamilton, 1971
	4.8	Inhibition of ADPG pyrophosphorylase	Kanapka, Khandelwal, and Hamilton, 1971
		Inhibition of ADPG glucan transferase	Kanapka, Khandelwal, and Hamilton, 1971
	9.6	Inhibition of phosphoglucomutase	Kanapka, Khandelwal, and Hamilton, 1971
Escherichia coli	50	Inhibition of phosphatases that hydrolyze α-methyl-D-glucose-6-P; inhibition strong at acid pH, but no inhibition at alkaline pH; in vivo phosphorylation and dephosphorylation inhibited at acid pH	Haguenauer and Kepes, 1972
Salivary sediment mixtures	1.0	Stimulation of CO_2 formation from glucose at acid pH	Sandham and Kleinberg, 1973
	≤0.22	No effect on CO_2 formation	Sandham and Kleinberg, 1973
	0.22, 1.0	Inhibition of lactic acid and total acid production	Sandham and Kleinberg, 1973

TABLE 3.11 (continued)

Organism	Concentration (m*M*)	Effect	Reference
Entamoeba histolytica	25	Blocking of glycolysis; no effect on initial transport rate of 10 m*M* 3-*O*-methylglucose	Serrano and Reeves, 1974
Bacillus stearothermophilus	*a*	Inhibition of α-methyl-D-glucoside accumulation in presence of ATP and Embden-Meyerhoff-Parnas pathway intermediates; fluoride plus sodium azide almost completely inhibited transport of α-methyl-D-glucoside with NADH as cosubstrate	O'Leary, Busta, and McKay, 1975
Streptococcus mutans	47.5	Inhibition of sugar uptake, partially alleviated by potassium; potassium extruded from both fermenting and nonfermenting cells — accompanied by rapid F^- uptake by fermenting cells, then immediate release of F^- back into the medium; intracellular sugar changes slight; complete inhibition of acid production	Luoma and Tuompo, 1975

[a] Fluoride concentration not given.

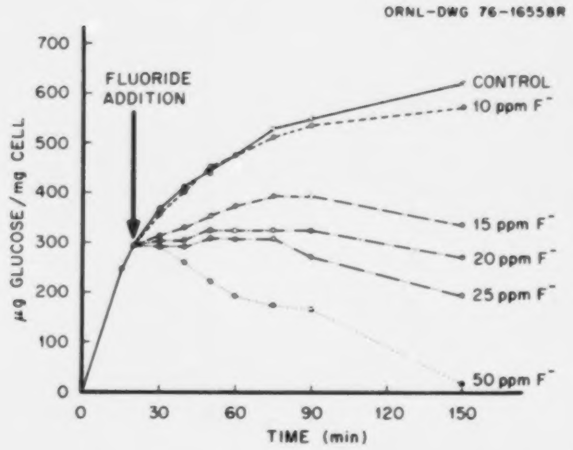

Figure 3.7. Effect of fluoride on an actively synthesizing polysaccharide system of *Streptococcus mitis*. Source: Adapted from Weiss et al., 1965, Figure 3, p. 842. Reprinted by permission of the publisher.

concentrations of 15 ppm and higher on glucose incorporation. At 50 ppm fluoride, bacterial incorporation of sugar ceased about 2 hr after fluoride addition and degradation of intracellular polysaccharide occurred. Sodium monofluorophosphate (Na_2PO_3F) was also tested and was found to be less inhibitory to polysaccharide synthesis than sodium fluoride. Inhibition of polysaccharide synthesis, especially synthesis of capsular dextrans and levans, also occurred in the cariogenic bacterium *S. mutans* (Bowen and Hewitt, 1974). The production of dextran (polyglucan) and levan (polyfructan) was altered by 70 ppm fluoride in five different strains of *S. mutans*. The ratio of fructose to glucose was significantly higher in cultures grown in the presence of 70 ppm fluoride than in control cultures grown in the absence of fluoride. However, Schachtele (1977) was unable to show inhibition of various forms of the enzyme glucosyl transferase, even at fluoride concentrations as high as 26.3 m*M*. He also indicated a high level of resistance for other enzymes involved in metabolism of dextrans and levans.

Yost and VanDemark (1978) found that *Leuconostoc mesenteroides*, which does not transport sugars by the phosphoenolpyruvate-requiring phosphotransferase system, was less sensitive to fluoride in terms of growth inhibition than *S. mutans*, which does have the phosphotransferase system.

In addition, Kashket, Rodriguez, and Bunick (1977) found that very low levels of fluoride inhibit glucose utilization by oral streptococci and do not have any stimulatory action. The whole subject of the effects of fluoride on carbohydrate uptake and metabolism was reviewed by Hamilton (1977).

3.3.2.3 Protein Synthesis and Nucleic Acid Metabolism — Of the fluorinated compounds that can alter protein synthesis and nucleic acid metabolism of microorganisms, the substrate analogs *p*-fluorophenylalanine (*p*-FPhe), 5-fluorodeoxyuridine (FdUrd), and 5-fluorouracil (FU) have been the most widely studied. Their incorporation into biopolymers and the resulting metabolic effects were reviewed by Heidelberger (1972) and Fowden (1972).

Mandel (1969) extensively reviewed data on the incorporation of FU into RNA. Table 3.1 (Section 3.2.1) shows that 5% to 80% of the original uracil in several bacterial and fungal species and viruses can be replaced by FU. In most cases the incorporation could be reversed by the addition of uracil. Subcellular effects of FU replacement of uracil included alterations in ribosomal composition and properties; changes in the functions, properties, and base composition of transfer RNA (*t*RNA); and modifications in the functions of messenger RNA. Recently, more work has been done using *E. coli* B *t*RNA. Lowrie and Bergquist (1968) obtained up to 100% replacement of uracil by FU in *t*RNA, and Kaiser (1969, 1971) obtained 80% to 84% replacement. Lowrie and Bergquist found that the secondary structure of FU-*t*RNA was altered. However, Kaiser (1971) reported that fluorouridine replacement of uridine and uridine-related minor base components had only minor effects on secondary and tertiary properties, especially in the presence of Mg^{2+}. The FU-*t*RNA retained about 90% of its ability to accept amino acids (Kaiser, 1969).

Cohen et al. (1958) determined that the inhibitory effect of FdUrd on DNA synthesis in *E. coli* was due to the inhibition of thymidylate synthetase by the phosphorylated 5'-monophosphate of FdUrd. In this case, the fluorinated pyrimidine is not incorporated into the nucleic acid but rather prevents the synthesis of a component (thymidylate) essential for DNA replication.

The enzyme thymidylate synthetase can be inhibited in some microorganism cells by the fluorinated base 5-fluorocytosine. The major source of deoxythymidylate for DNA synthesis is thus suppressed. Inhibition appears to result from formation of 5-fluorodeoxyuridylate which can irreversibly bind to thymidylate synthetase to inactivate it. The kinetics of the reaction with the enzyme from *Lactobacillus casei* were described (Danenberg and Danenberg, 1978).

Mandelbaum-Shavit and Kisliuk (1978) investigated mutants of *Pediococcus cerevisiae* resistant to FdUrd. They defined two types: one with reduced or absent thymidylate kinase activity, which is thought to be involved in thymidine uptake by bacterial cells; and the other with impaired permeability to the fluoronucleotide but with normal kinase activity.

5-Trifluoromethyl-2'-deoxyuridine (F_3Thd) at 1 *μM* irreversibly inhibited replication of vaccinia virus (the W.R. neurotropic strain) in HeLaS3 cell cultures (mycoplasma-free) with selective toxicity to the virus (Umeda and Heidelberger, 1969). FdUrd (1 *μM*) did not selectively inhibit the virus. Its inhibition was transient, occurring primarily on the first day. Umeda and Heidelberger concluded that the major antiviral activity of F_3Thd was probably due to its incorporation into viral DNA.

The incorporation of *p*-FPhe into microbial protein can cause many diverse effects. When incorporated into *E. coli* B 15_{T^-} it blocked cell division; it also blocked ribonucleic acid polymerase synthesis when thymine was added at the same time to thymine-starved cultures (Hardy and Binkley, 1967). In *E. coli* the interference with cell division seemed to be with the initiation of DNA replication. Other consequences of *p*-FPhe incorporation into microbial protein were discussed by Fowden (1972). These include alterations in shape and size of cells, interference with cell differentiation, and alterations of eukaryotic chromosomes.

The fluorinated base 5-fluorocytosine, usually in combination with amphotericin B, has been used for many years for treatment of infections caused by yeasts of the genera *Candida*, *Torulopsis*, and *Cryptococcus*. The mode of action in *Candida* appears (Polak and Scholer, 1975) to involve metabolic conversion to 5-fluorouridylate, which is then incorporated into RNA with up to 50% replacement of uracil. The replacement then leads to disturbances in protein synthesis. There was no indication of conversion to 5-fluorodeoxyuridylate in *Candida* or for inhibition of thymidylate synthetase.

An uncoupler of photophosphorylation, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) at 1.5×10^{-7} *M* inhibited replication of the virulent bacteriophage RC1 in the nonsulfur purple photosynthetic bacterium *Rhodopseudomonas capsulata* strain Z-1 (Figure 3.8) (Schmidt, Yen, and Gest, 1974). The growth rate of the uninfected control plus 1.5×10^{-7} *M* FCCP was substantial, but the infected cells in the presence of FCPP did not

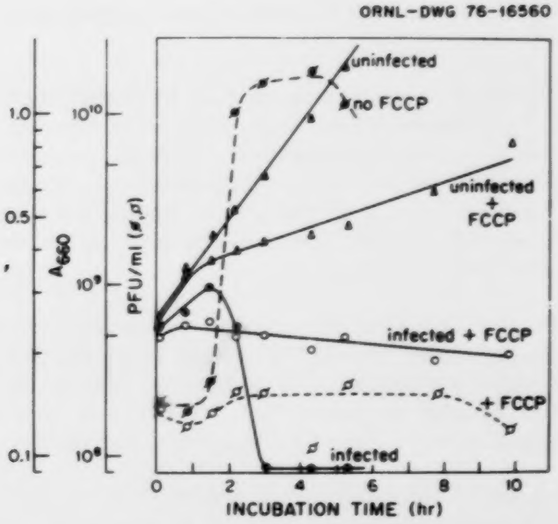

Figure 3.8. Effect of the phosphorylation uncoupler carbonylcyanide-*p*-trifluoromethoxy-phenylhydrazone (FCCP) on replication of RC1 phage in photosynthetically grown cells of *Rhodopseudomonas capsulata* Z-1 incubated under photosynthetic conditions. Source: Adapted from Schmidt, Yen, and Gest, 1974, Figure 4, p. 235. Reprinted by permission of the publisher.

show production of progeny phage. The phage requirement for energy was more stringent than that of the bacterial host cell.

Inorganic fluoride can inhibit several reactions involving nucleic acids. Effects of sodium fluoride (50 m*M*) on *N. crassa* (protein, RNA, and DNA synthesis reduction) are illustrated in Figure 3.9 (Timberlake and Turian, 1975). The cultures used were submerged pregerminated conidia. Leucine incorporation into protein was the most rapidly reduced. Fluoride inhibits DNA polymerase activity in the presence of Mg^{2+} (Hellung-Larsen and Klenow, 1969; Lehman et al., 1958). Hellung-Larsen and Klenow reported that 0.16 *M* potassium fluoride caused 90% inhibition of DNA polymerase of *E. coli* B. They correlated inhibition with the formation of precipitable complexes containing Mg^{2+}, substrate phosphatases, and fluoride.

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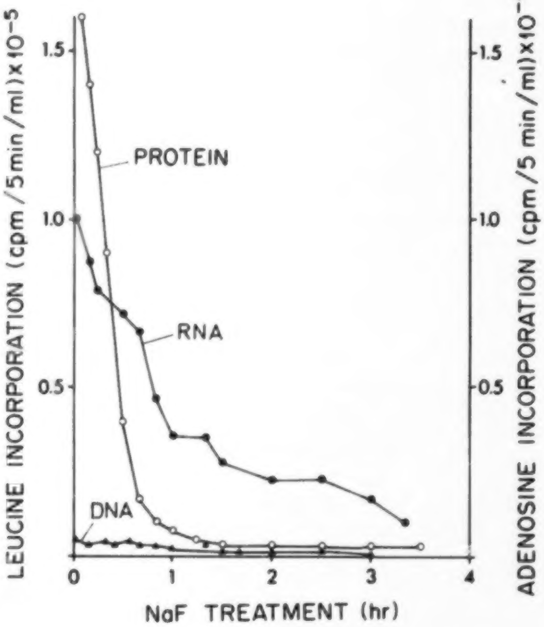

Figure 3.9. Effect of NaF treatment on protein, RNA, and DNA synthesis of *Neurospora crassa*. Source: Adapted from Timberlake and Turian, 1975, Figure 4, p. 153. Reprinted by permission of the publisher.

Fluoride may have important effects on nucleotide metabolism. For example, Khandelwal and Hamilton (1971) found that fluoride stimulated the adenyl cyclase enzyme of *S. salivarius*, and it is possible that this action may have important effects on the regulatory circuits of microorganisms. Marquis (1977) found that fluoride inhibited the membrane ATPase of *S. faecalis* and suggested that this action could be partly responsible for the potassium deficiency of cells treated with fluoride.

3.3.2.4 Enzyme Activities — Inhibition of enzymes responsible for acid production may be especially important in the study of cariogenesis (Jenkins, 1970). The enzyme of the Embden-Meyerhof pathway most sensitive to fluoride is enolase (Jenkins, 1970; Warburg and Christian, 1942), although the actual site of enzyme inhibition in saliva seems to be one involving glucose uptake by the cell (Jenkins, 1970) and ionic transport (Luoma and Tuompo, 1975).

Effects of fluorides on a variety of microbial enzyme systems are given in Tables 3.12 and 3.13. In general, varying degrees of inhibition are indicated, although the respiration and rate of multiplication of certain yeasts are enhanced. Table 3.14 indicates no inhibitory effects of fluoride (up to 9.6 m*M*) on the activity of several enzymes involved in the synthesis and degradation of glycogen in crude extracts of *Streptococcus salivarius* (Kanapka, Khandelwal, and Hamilton, 1971). Additional information concerning fluoride effects on carbohydrate metabolism is in Section 3.3.2.2. Table 3.15 lists some effects of 5-fluorouracil (FU) on various microbial enzymes (Mandel, 1969). The inhibitory action of FU seems to involve the fidelity of protein synthesis on FU-substituted mRNA.

TABLE 3.12. ENZYME INHIBITION BY SOLUBLE FLUORIDES

Organism	Enzyme system	Effect[a]
Bacidiomycetes	Acid phosphomonoesterase	+++
Yeast	Adaptive fermentation of galactose	++
	Cell permeability	+
	Fermentation	++
	Glycogen storage	++
	Multiplication	--
	Respiration	++
Yeast top	Respiration	--
	Acetycholinesterase	?
	Apozymase + cozymase	+
	Cytochrome oxidase	0
	Glucosulphatase	++
	Invertase	0
	Phosphatase (in vitro)	+
	Phosphatase (in vitro) reactivation	+++
	Succinodehydrogenase	+++
	Takadiastase on glycerophosphates	++
	Takasulphatase	+++

[a] 0 indicates no effect; + to +++ indicates varying degrees of inhibition; - to -- indicates promotion.

Source: Adapted from Eagers, 1969, Table 16, pp. 107-108. Data collected from several sources. Reprinted by permission of the publisher.

TABLE 3.13. EFFECTS OF FLUORIDE ON MICROBIAL ENZYMATIC ACTIVITY

Enzyme	Source	Compound	Concentration	Effect	Reference
Inorganic pyrophosphatase	*Ferrobacillus ferrooxidans* (*Thiobacillus ferrooxidans*)	KF	10^{-2} *M*	90% inhibition	Howard and Lundgren, 1970
β-Galactosidase	*Escherichia coli*	*p*-Toluenesulfonyl fluoride	Enough to inhibit protein breakdown	Blocked induction in starving cells	Goldberg, 1971
		Phenylmethane sulfonyl fluoride	300 μg/ml	Blocked induction in starving cells; slight inhibition in cells growing on glycerol	Goldberg, 1971
Fungal lactase A and B	*Polyporous versicolor*	F^-	1 equivalent	Almost complete inhibition when bound to type 2 Cu^{2+}; inhibition decreased in presence of substrate	Brändén, Malmström, and Vänngard, 1973
p-Nitrophenylphosphatase	*Streptococcus mutans*	NaF	0.67–1.67 m*M*	No repressive or inducive effect	Knuuttila and Mäkinen, 1973
Phytase	*Pseudomonas* sp.	F^-	1.0–8.0 m*M*	Inhibition varied as the cube of fluoride ion concentration	Irving and Cosgrove, 1971

TABLE 3.14. INFLUENCE OF SODIUM FLUORIDE ON THE ENZYMES INVOLVED IN THE SYNTHESIS AND DEGRADATION OF GLYCOGEN IN CRUDE EXTRACTS OF *STREPTOCOCCUS SALIVARIUS*

Enzyme	Activity of enzyme at NaF (mM) concentration					
	0	1.2	2.4	4.8	7.2	9.6
Phosphoglucomutase						
Preincubation	147[a]	159	171	147		171
No incubation	147	159	147	147		147
ADPG pyrophosphorylase	87[b]	90	97	85		
ADPG glucan transferase	106[c]	107	111	108		
Phosphorylase	235[d]	363	407		450	

[a] Nanomoles glucose-6-P formed per milligram protein per minute.

[b] Picomoles ADP-glucose formed per milligram protein per minute.

[c] Nanomoles glycogen formed per milligram protein per minute.

[d] Picomoles glucose-1-P incorporated into glycogen per milligram protein per minute.

Source: Adapted from Kanapka, Khandelwal, and Hamilton, 1971, Table II, p. 600. Reprinted by permission of the publisher.

TABLE 3.15. EFFECTS OF 5-FLUOROURACIL INCORPORATION ON ENZYMATIC ACTIVITY

Enzyme	Source	Effect	Comments
β-Galactosidase	*Escherichia coli*	Inhibition	Inducible and constitutive; occasionally reversed by uracil; decreased enzyme specific activity
Succinate dehydrogenase	*E. coli*	Stimulation	Constitutive
Catalase	*E. coli*	None	Constitutive
D-Serine dehydrase	*E. coli*	Inhibition	Inducible
β-Glucuronidase	*E. coli*	Inhibition	Constitutive (?)
Serine deaminase	*E. coli*	None	
Glucose-6-phosphate dehydrogenase	*E. coli*	None	
Alkaline phosphatase	*E. coli*	None	Decreased thermostability
Ribonuclease	*Staphylococcus aureus*	Inhibition	Inconclusive
Deoxyribonuclease	*S. aureus*	None	Inconclusive
Ribonuclease	*Bacillus subtilis*	Inhibition	Exoenzyme
Alkaline phosphatase	*B. subtilis*	Inhibition	Inducible
α-Amylase	*B. subtilis*	None	Exoenzyme; thermostability unchanged
Aconitase	*Bacillus cereus*	Inhibition	Constitutive
Malate dehydrogenase	*B. cereus*	Inhibition	Constitutive
Fumarase	*B. cereus*	Inhibition	Constitutive
Succinate dehydrogenase	*B. cereus*	None	Constitutive
NADH oxidase	*B. cereus*	None	Constitutive
Penicillinase	*B. cereus*	None	Inducible
d-Aminolevulinate synthetase	*Rhodopseudomonas spheroides*	Inhibition	Uracil reversed effect
Succinate dehydrogenase	*R. spheroides*	None	
α-Glucosidase	*Saccharomyces carlsbergensis*	None	Inducible; no qualitative change
Galactosidase	*S. carlsbergensis*	None	Inducible; no qualitative change

Source: Adapted from Mandel, 1969, Table 6, p. 117. Data collected from several sources. Reprinted by permission of the publisher.

3.4 PROSPECTS FOR FUTURE RESEARCH

3.4.1 Inorganic Fluorides

3.4.1.1 Dental and Medical Uses — It seems unquestionably clear that there will be extensive future use of fluorides as anticaries agents, and since they do have antiplaque properties, possible use of them against periodontal disease. A study by Erickson (1978) of mortality rates in 24 cities with fluoridated water and 22 with nonfluoridated water revealed no significant differences between the two. Therefore, the view that water fluoridation has no harmful effects was supported. These findings are encouraging for more extensive use of water fluoridation in the United States and other industrialized countries. Of course, this increased use means greater exposure of microorganisms, as well as humans, to fluorides.

There is currently an increasing incidence of dental caries, even in developing countries, mainly because of changes in diet. This increase has prompted study by the World Health Organization and others. The use of fluoride seems to be the best way to curb this rise in caries. However, in Third World countries water fluoridation is not always feasible, and fluoride may have to be provided in foods or in tablets. Again, the future seems to hold increased fluoride exposure to people and flora.

Topical fluorides are likely to become more popular in the United States. In fact, there are new fluoride-containing mouthwashes on the market in the United States with approximately 23 millimoles of fluoride. Of course, topical fluoride preparations used under supervision of a dentist are much more concentrated and cannot reasonably be used by the general public because of the danger of accidental ingestion. However, the preparations appear to be so highly effective that their use will become more widespread. It is not now clear whether or not it is best to have a prophylactic cleaning of the teeth prior to topical fluoride application. The National Institute of Dental Research is currently funding work on the question. There is some feeling that leaving bacteria in plaque on teeth helps in fluoride retention.

Fluoride usage in medicine is not extensive; however, fluoride is experimentally used to reduce osteoporosis. The maximum dosage is usually about 50 mg/day given with calcium and vitamin D. If distributed evenly throughout the body, this dosage would give a level of only about 0.03 m*M*. One would not expect this concentration of fluoride to greatly affect the normal flora. However, if fluoride is accumulated by the gut bacteria, it is possible that microbiologically significant levels could be attained. Some 10% of fluoride ingested is excreted in feces.

Microbiological aspects of fluoride ingestion by man have been sketchily studied, and concerns about the safety of increased ingestion require more thorough investigations. It has been clearly shown that bacteria in dental plaque accumulate rather high levels of fluoride (millimolar range). These levels are effective in upsetting microbial physiology, even to the extreme response of lysis shown by certain species of bacteria. The concentration of fluoride by plaque would be expected to have effects on the population, favoring the less sensitive types. Indirect effects would also be expected. For example, fluoride reduces acid production by plaque, and less acid conditions should favor growth and metabolism of the less aciduric members of the flora. Reductions in acid production due to fluoride ingestion could have effects in other parts of the body, for example, in the vagina where the maintenance of acid conditions through bacterial metabolism is thought to be partly responsible for resistance to certain infections.

There is a possibility that the now long-term use of fluoride in drinking water has contributed to a rise in resistant bacteria, much as the use of antibiotics has led to a disasterous selection of resistant bacteria. There is a need to find out if the use of fluoride does indeed result in selection of resistant forms, and if the selection occurs in the normal flora of man.

Another possible effect of fluoride on normal flora that is being investigated deals with the natural immune systems in the mouth — specifically lysozyme, lactoperoxidase, and lactoferrin. It appears that bacteria grown in media with plaque levels of fluoride are more susceptible to lysozyme killing or inactivation by the lactoperoxidase system than are bacteria from nonfluoridated media. Fluoride may adversely affect bacteria indirectly by rendering them more susceptible to normal host defense mechanisms. An interest in this research comes from the desire to formulate more effective anticaries regimens.

The National Institute of Dental Research has a number of contracts to assess the interactions of fluoride with other ions and their effects on dental caries. There is also a need for research on interactions of fluoride with other agents used to reduce caries (e.g., chlorhexidine, calcium phosphate rinses, and alkylamines).

3.4.1.2 Basic Studies of Microbial Responses to Fluoride — The National Institute of Dental Research has tried to obtain basic information on the mechanism of the anticaries action of fluoride. A large component of the needed information is microbial responses to fluoride. Recent research has shown that bacteria have a complex array of responses to fluoride.

Enolase-action inhibition is the most widely accepted view of how fluoride inhibits bacterial acid production. This inhibition results in diminished supplies of phosphoenolypyruvate and secondary inhibition of the phosphotransferase sytem for sugar uptake. However, it appears that fluoride may also act as a transmembrane proton conductor to acidify cytoplasm and render cells acid sensitive. The importance of the latter action in the inhibition of the metabolism of oral bacteria remains to be determined.

There are indications that fluoride is effective in either inhibiting or changing the production of extracellular polysaccharides by oral bacteria. Since the polymers are thought to play important roles in colonization of teeth by bacteria, this action may be involved in the anticaries effects of fluoride.

The uptake of fluoride by bacteria, especially in relation to fluoride concentration by dental plaque, is not completely understood. It is not known if specific transport systems bring fluoride into the cell or if fluoride enters only by diffusion of the hydrogen fluoride form. Researchers do not know if the bacterial membrane has a low level of permeability to the anionic form or what polymers are responsible for binding fluoride in the cell. There is still a question of whether this tightly bound fluoride is of any metabolic significance.

Fluoride has a number of other important actions that may be pertinent to its effects on plaque bacteria. For example, it inhibits the membrane ATPase of streptococci, and presumably other bacteria. Perhaps this inhibition causes sufficient loss of potassium from cells so that processes such as glycolysis and protein synthesis are inhibited. There may be other metabolic actions of fluoride not currently known. Certainly, many isolated enzymes are inhibited by fluoride, and a multiplicity of actions are expected within the cell. Also, some enzymes (e.g., adenylate synthetase) can be stimulated by fluoride. Again, important metabolic alterations result.

Another area likely to receive a great deal of attention is related to the anticaries action of topical fluorides. There is evidence to suggest that the action may be different from the anticaries action of fluoride in drinking water. It is clear that fluoride, especially in concentrated solutions, can kill certain bacteria. However, the mechanism of this killing and how the killing alters plaque flora are not known.

Major research during the next decade on the biological actions of fluoride will probably have a dental orientation. However, there are many industrial and environmental problems that should receive attention, some relating to the use of fluoride as an anticaries agent. For example, water used for microbial processes (e.g., brewing or sewage treatment) is fluoridated in many cases, and fluoride may be concentrated by the organisms with resulting alterations in metabolism. The major industrial problem probably comes from emission of fluorides from industrial plants with resultant pollution of surrounding areas. The major sector of the biological community interacting with these fluorides would be microorganisms. These microorganisms would metabolize the compounds, and to date there is little information on the specifics of this process.

3.4.2 Organic Fluorocompounds

3.4.2.1 New Compounds — One major area of future research in the microbiological aspects of organic fluorides is the development of antimicrobial agents. The most useful compounds should be selectively toxic to microorganisms. Microbial resistance to antibiotics is increasing at an alarming rate, and there is an imminent need for compounds that can be used in place of, or in conjunction with, antibiotics. For example, fluorocytosine is commonly used with amphotericin B in treating fungal infections caused by organisms such as *Cryptococcus neoformans*. Recently, a new fluoroderivative, flumequine, was introduced as a substitute for nalidixic acid in the treatment of urinary tract infections.

Because of its size, fluoride is a good substitute for hydrogen in organic compounds, and fluoroanalogues are commonly active biologically. For example, many fluoroamino acids are incorporated into proteins in place of the natural compounds. It seems that a rational approach would be to synthesize fluoroderivatives of metabolites peculiar, or nearly so, to prokaryotes. For example, peptidoglycan is a compound that occurs only in the bacterial cell wall. Many peptidoglycans contain the unusual amino acid diaminopimelate. Perhaps a fluoroderivative of diaminopimelate would have selective toxicity for prokaryotes. Nearly all peptidoglycans contain D-amino acids; for example, fluorinated D-alanyl-D-alanine might be selectively toxic. Certainly, it seems that imaginative research in this area is likely to be profitable. Development of antiviral properties and further testing of compounds is likely to yield positive results.

Much research is still to be done in developing fluoroderivatives for use in basic biochemical studies. Again, fluoride tends to mimic hydrogen as a substituent, and fluoroanalogues tend to be active biologically. Fluoride is currently a popular substituent because of the possibility of analyzing ^{19}F nmr signals. This field too seems to be one in which imaginative research is bound to be successful.

3.4.2.2 Microorganisms for Degradation of Fluoroorganics — Microorganisms are able to catabolize many fluoroorganic compounds, sometimes resulting in cleavage of the carbon-fluoride bond. These organisms should be useful in cases of dangerous pollution by organic fluoride compounds. Moreover, organisms capable of degrading fluoroorganics would be of interest to biochemists studying reaction mechanisms and to microbiologists. Specific microbes could be isolated for use in specific pollution problems involving organic fluorides.

3.4.2.3 Additional Uses for Fluorinated Anesthetics — Recent evidence suggests that anesthetics, including fluorinated ones, can inhibit the growth of cells. The spectrum of inhibition spans all cell types, from bacteria to mammalian cells. Microorganisms should prove to be useful for basic studies of the mechanisms of growth inhibition, although it is unlikely that fluorinated anesthetics will find use as antiseptics or disinfectants.

SECTION 3

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SECTION 4

BIOLOGICAL ASPECTS IN PLANTS

Fluoride is a well-studied pollutant in the ecosystem, and considerable data exist on its effects on plant systems. For some of the earlier work in the field, several good literature reviews may be consulted (Adams, 1956; Brewer, 1966; Chang, 1975; National Academy of Sciences, 1971; Thomas and Alther, 1966; Treshow and Pack, 1970).

4.1 SUMMARY

The metabolism and effects of fluorides in plants have been well studied, but important information is still lacking. Although soils contain a high fluoride content (10 to 1000 ppm), most is in a form not absorbed by plants. Acid soils tend to have a higher available fluoride content; liming decreases this availability. The levels of major nutrients in the soil affect the amount of fluoride absorbed through the root. Fluoride is translocated from root to shoot, and fluoride in the leaf moves to the tips and edges of the leaves but not out of the leaves.

In polluted areas, most fluoride is probably absorbed by leaves. Leaves of plants found in the vicinity of known sources of atmospheric fluoride pollution, such as aluminum and phosphate fertilizer industries, have high fluoride concentrations. Fluoride fumigation at night, when stomates are closed, produces less accumulation of fluoride in tissues than fumigation during the day. Little downward translocation of fluoride from shoots to roots has been found. Gaseous fluorides are more effectively absorbed than particulate fluorides and lead to greater injury. Cryolite, even when present at high levels, produces few injury symptoms in plants.

Fluoride metabolism in plants is not well understood. Some plants have the ability to synthesize relatively large quantities of carbon-fluorine compounds. These plants are toxic to animals, and the toxic principle is usually monofluoroacetate. Whether all plants can synthesize carbon-fluorine bonds is uncertain. There is some evidence that a variety of plants can form small amounts of fluoroacetate and fluorocitrate. No information is available on the enzyme system(s) necessary to synthesize or degrade the organofluorides. Why fluoroacetate is not toxic to these plants is not known.

Fluoride is found in virtually all plants. In unpolluted areas, fluoride concentrations range from about 2 to 20 ppm. Except for certain species (e.g., *Camellia*), plants are not able to concentrate fluoride much above these levels from the soil. There is no apparent relationship between soil concentration of fluoride and tissue concentration of fluoride. However, exposure to even low concentrations of atmospheric fluoride can lead to significant accumulation of foliage. Significant amounts of fluoride can be removed from leaves by washing with distilled water or detergents. Tissue fluorides increase as length of exposure and atmospheric concentrations increase. Data are inadequate to determine subcellular sites of localization; however, some evidence suggests that chloroplasts accumulate fluoride to a greater extent than mitochondria or cell walls.

Fluoride is eliminated from plants by loss of leaves, twigs, and roots; by rain; and, although evidence for this is meager, perhaps through volatilization of organofluorides.

Fluorides affect cells in an undefined manner. Eventually, the altered metabolism of the cell is expressed as visible symptoms, which ultimately can cause death of the plant or plant organ. The buildup of fluorides in plant material increases the availability of fluoride to other components of the ecosystem, which may, for example, increase the incidence of fluorosis in animals.

Although fluorides are known to be in vitro inhibitors of a variety of enzymes, it is difficult to establish whether inhibition of these enzymes also occurs in vivo and which inhibition is directly responsible for the altered metabolism. It is known, however, that fluorides alter photosynthesis, carbohydrate metabolism, respiratory and oxidative processes, RNA metabolism, and calcium nutrition.

Fluoride injury to foliage usually involves tip or marginal necrosis, sometimes preceded by chlorosis; however, other environmental stresses can duplicate these symptoms. Wilting is observed with higher doses of fluorides. Not all plants are equally sensitive to fluorides; gladiolus, apricot, and Douglas fir are examples of sensitive plants, whereas cherry, tomato, and wheat are resistant plants. Fruits can also be injured by fluoride exposure. The most common example of injury is the soft suture disease in peaches; fruit yield can be decreased by fluoride exposure. High fluoride concentrations in solutions can inhibit germination of seeds and restrict growth, although at low concentrations some enhancement of linear growth may occur. Growth inhibition is usually accompanied by visible injury symptoms, but hidden injury (growth inhibition without visible injury) has been reported when plants were exposed to relatively high concentrations of fluoride.

From an agricultural point of view, permissible fluoride exposures are difficult to determine. Interacting factors include the species diversity of the community, the sensitivity of each species, and the nature and pattern of fluoride exposure. Data are insufficient to recommend air quality concentrations at the present time. Few data also exist on the effects of interactions of fluoride with other pollutants or with parasitic organisms.

Fluorides apparently can be mutagenic; cytological abnormalities have been observed in a variety of plants exposed to either hydrogen fluoride or sodium fluoride.

4.2 METABOLISM

Fluorine, a rather abundant element, occurs naturally in all soils. It is released to the environment by a number of industrial processes. Fluoride contamination of plants has been extensively investigated because exposure to fluorides can induce disease. Although greater emphasis has been placed on symptomatology, there is considerable information concerning the metabolism of fluoride in plants. However, information gaps do exist, particularly with respect to the fate and effect(s) of fluoride at the cellular and molecular level.

Based on the generally accepted criteria of Arnon and Stout (1939), fluoride is not considered essential for plant nutrition. Although some reports show that in certain plants (Douglas fir, citrus, rose, alfalfa, and beans) fluoride can stimulate growth, this increased growth rate does not normally result in a better adapted plant (Thomas and Alther, 1966).

4.2.1 Uptake and Absorption

Because fluoride can be absorbed by most plant organs, the major route of entry depends on the source of fluoride. For example, atmospheric pollution from aluminum and phosphate industries is the major source of fluoride for many plants, entry occurring through stems and leaves. Soils, however, contain considerable amounts of fluoride, and absorption from soil occurs if the fluoride is in an available state. No information was found on the uptake mechanism for any cell type.

4.2.1.1 Exposure to Fluoride in Soil — Most soils contain from 10 to 1000 ppm fluoride. In alkaline soils, much of this is bound in clays, but in acid soils some fluoride is soluble and can be absorbed by the plant.

Sand and water culture experiments with added sodium fluoride show that plant roots will absorb fluoride, the amount absorbed depending on external concentration and exposure time. For example, after addition of isotopically labeled sodium fluoride to soil, fluorine-18 was detected in roots, stems, and leaves of tomato plants (*Lycopersicon esculentum*), indicating both absorption and translocation (Ledbetter, Mavrodineanu, and Weiss, 1960). Furthermore, uptake of fluoride by whole grass seedlings grown in water containing 5.25 to 21.00 m*M* fluoride increased from 28 to 51 ppm (wet weight) after 1-hr exposure, from 30 to 62 ppm after 4-hr exposure, and from 58 to 282 ppm after 16-hr exposure (Peters and Shorthouse, 1964). Plants grown in 1.05 m*M* fluoride contained only 3.4 ppm after a 4-hr exposure.

Acacia georginae grown in water culture and then exposed for 18 hr to an aqueous solution containing 10 ppm fluoride accumulated 3.6 ppm inorganic fluoride (wet-weight basis) in the aerial parts and 10.3 ppm in the roots (Peters, Murray, and Shorthouse, 1965). When exposed to 20 ppm fluoride for 120 hr, the inorganic fluoride in the aerial parts increased from 1.2 to 5.5 ppm, and the root content increased from 1.4 to 16.2 ppm. With higher concentrations of fluoride in the growth solution (200 to 300 ppm), large amounts of fluoride were taken up (Figure

4.1). Considerable variation among plants was observed in these experiments. For example, four plants grown in water culture and exposed to 10 ppm fluoride for eight days contained from 10 to 25.2 ppm fluoride. In pot experiments where fluoride was supplied as a saturated solution of calcium fluoride for 54 days, the aerial portions contained from 0 to 82 ppm inorganic fluoride.

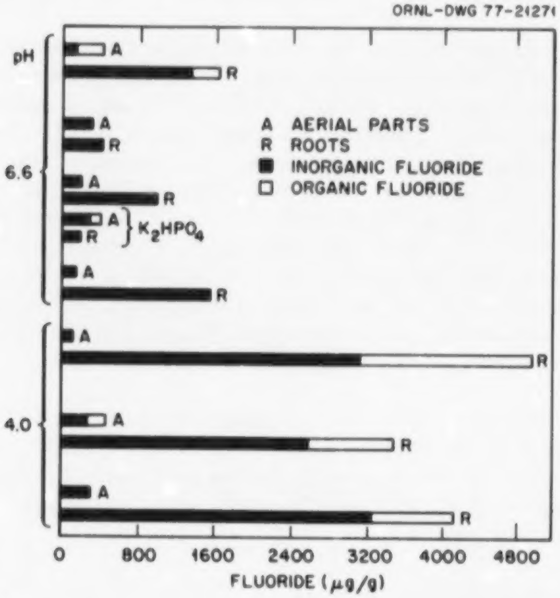

Figure 4.1. Comparison of the amounts of fluoride taken up by *Acacia georginae* from solutions of sodium fluoride, 300 μg/ml (15.75 m*M*) at pH 6.6 and pH 4.0 (acidification with nitric acid). Experiment 3 was also exposed to dipotassium hydrogen phosphate. Source: Adapted from Peters, Murray, and Shorthouse, 1965, Figure 2, p. 727. Reprinted by permission of the publisher.

Liming soil decreases the available fluoride content. Studies by MacIntire et al. (1949) showed that tops of plants do not accumulate fluoride from soils amended with calcium fluoride, rock phosphate, or hydrogen fluoride when the soils were adequately limed. In further studies, MacIntire et al. (1951) increased the fluoride content of clover by adding hydrofluoric acid to Hartsells fine sandy loam and Clarksville silt loam. Treating the soil with lime reduced the fluoride content of clover to control values (about 20 ppm). Similarly, Hansen, Wiebe, and Thorne (1958) reported that sodium fluoride additions to soil increased the plant-fluoride content of turnips and alfalfa grown in Orem loamy soil (low lime, low clay, and low organic content) to a much greater extent than those grown in soils higher in lime or clay. Additions of sodium fluorosilicate produced similar results, although less fluoride was absorbed than with sodium fluoride additions. In other studies, increasing the pH of loam and sandy loam soils supplemented with fluoride from 4.5 to 6.5 decreased the amount of fluoride in the leaves of tomato and buckwheat (Prince et al., 1949). Similar percentage decreases in leaf fluoride concentration were observed with either hydrogen fluoride or sodium fluoride added to Sassafrass loam or to sandy loam soils. Hurd-Karrer (1950) found that liming decreased the uptake of fluoride in collards, buckwheat, and barley. In unlimed plots with added sodium fluoride, uptake was greater from loamy sand soils than from sandy loam soils.

Because soil fluoride may be unavailable to plants, a direct relationship between soil fluoride content and plant fluoride content does not necessarily exist. However, Israel (1974*a*) found a positive statistical correlation between soil fluoride content and alfalfa fluoride content. Under his experimental conditions, a soil concentration of 120 ppm fluoride was necessary to produce the same tissue level of fluoride (1 ppm) as is produced by exposure to only 0.007 $\mu g/m^3$ of hydrogen fluoride. These data illustrate the difficulty of trying to correlate tissue fluoride content with soil fluoride content in field studies where small, but measurable, atmospheric concentrations of fluoride may be present. These data also support the argument that airborne fluorides do not add enough fluoride to soils to increase plant uptake from the soil.

In a German industrial area, yearly additions to soil by fallout from the atmosphere were approximately 2.1 kg of fluoride per hectare, and additions by fertilization were between 8 and 20 kg of fluoride per hectare (Oelschlager, 1972). Of this amount, only about 0.1% to 0.4% is removed by harvesting crops.

Levels of nutrients in the soil can also affect the amount of fluoride taken up by plants. The data of Brennan, Leone, and Daines (1950) (Table 4.1) suggest that levels of nitrogen, calcium, and phosphorus affect the concentration of fluoride in tomato roots and leaves when fluoride is supplied to soil as sodium fluoride. However, the fluoride concentrations in roots and leaves are not greatly affected when fluoride is supplied in gaseous form (hydrogen fluoride) to the plant. On the other hand, MacLean et al. (1969) found an effect of mineral nutrition on the response of tomato plants to gaseous hydrogen fluoride. Exposure to hydrogen fluoride increased the extent of foliar injury in plants grown in either a calcium-deficient or magnesium-deficient medium. Fluoride uptake was greater in plants grown on potassium-deficient medium and was less in plants grown on calcium- or magnesium-deficient media. McCune, Hitchcock, and Weinstein (1966) found that nutrient levels did not affect fluoride accumulation in gladiolus, but the extent of necrosis was affected. Low levels of nitrogen or calcium reduced necrosis, whereas low levels of potassium, phosphorus, or magnesium increased necrosis. Necrosis was not affected by either iron or magnesium deficiencies. Pea plants grown in low-calcium solution showed more injury (reductions in number and weight of seeds) from fluoride fumigation than when grown in standard solution (Pack and Sulzbach, 1976). Pepper, however, showed the opposite response. Several types of mineral-deficient bean plants were tested, and only the nitrogen-deficient plants developed necrosis upon exposure to gaseous hydrogen fluoride (Adams and Sulzbach, 1961).

TABLE 4.1. EFFECT OF 50 ppm FLUORINE (AS NaF) IN NUTRIENT SOLUTIONS (SERIES A AND B) AND OF HF FUMIGATION (SERIES C AND D) ON THE DEGREE OF INJURY AND FLUORIDE LEVELS IN TISSUES OF TOMATO PLANTS GROWN WITH DIFFERENT LEVELS OF NITROGEN, CALCIUM, AND PHOSPHORUS

Nutrient	Concentration (ppm)	Series A (13 days treatment)			Series B				Series C (48 ppb HF, 4.5 hr)			Series D (470 ppb HF, 3.5 hr)		
		Degree of injury	Fluoride in tissue (ppm)		Degree of injury	Days of treatment	Fluoride in tissue (ppm)		Fluoride in leaves[a] (ppm)		Fluoride in roots, 1- and 7-day average (ppm)	Fluoride in leaves (ppm)		Fluoride in roots, 1- and 7-day average (ppm)
			Leaves	Roots			Leaves	Roots	1 day	7 days		1 day	7 days	
Nitrogen	14	None	74	280	None	31	250	1310	40	53	18	375	415[b]	19
	56	None	96	3010	Moderate	24	1000	3440	43	40	13	655	360[c]	13
	112	Moderate	540	2000	Moderate	13	500	1185	35	32	23	500	465[d]	22
	448	None	160	1800	None	31	430	1220	47	30	15	540	360[b]	16
Calcium	10	None	170	460	None	31	435	480	45	50	14	460	355[b]	15
	40	Moderate	950	1290	Moderate	6	1000	750	55	30	22	435	405[d]	32
	80	None	116	3610	Moderate	8	850	1300	40	40	18	550	255[c]	25
	240	None	62	4360	None	31	161	3945	45	30	14	405	255[b]	27
Phosphorus	0	Trace	274	2739	Trace	31	421	6585	43	40	30	385	420[b]	13
	0.8	Slight	480	2305	Slight	31	620	2305	40	40	11	375	365[b]	32
	15.5	Slight	464	1050	Moderate	13	890	2080	23	28	10	470	420[c]	17
	62	Moderate	1010	970	Severe	13	672	1340	40	35	14	575	280[d]	16

[a] No leaf injury in Series C.
[b] Slight leaf injury.
[c] Moderate leaf injury.
[d] Severe leaf injury.

Source: Adapted from Brennan, Leone, and Daines, 1950, Tables III, IV, and V, pp. 739-742. Reprinted by permission of the publisher.

Bovay (1969) observed that certain fertilizers containing boron produced symptoms of fluoride damage in crops. Field and pot studies demonstrated that apricot trees, grapevines, and forage plants took up more fluoride from boron-enriched fertilizers than from fertilizers without boron, even though the fluoride content in the two types of fertilizers was similar. About 57% of the fluoride in the boron fertilizer was fluoroborate. Apparently boron can increase plant uptake of fluoride.

4.2.1.2 Exposure of Plants to Fluoride in Air — It has long been recognized that exposure of plants to gaseous and particulate fluorides in the atmosphere results in fluoride accumulation and eventual injury (National Academy of Sciences, 1971; Thomas and Alther, 1966; Treshow, 1971).

The data of Hill (1969) illustrate the rate and extent of uptake of hydrogen fluoride by gladiolus leaves (Table 4.2). This general trend is observed by most researchers. Additional data on the relationships among length of exposure, air concentration, and tissue concentration of fluoride are presented in Section 4.2.4.

TABLE 4.2. CHANGES IN FLUORIDE CONCENTRATIONS IN DIFFERENT SECTIONS OF SNOW PRINCESS GLADIOLUS LEAVES FOLLOWING FUMIGATION WITH HYDROGEN FLUORIDE

Fluoride treatment	Time after beginning treatment	Fluoride content (ppm dry weight)		
		Tip 4 in.	Next 4 in.	Remainder of leaf
890 μg/m³ for 1 hr	2 hr	71	87	92
	5 hr	94	115	68
	45 hr	159	104	45
40 μg/m³ for 24 hr	1 day	132	97	34
	2 days	188	83	26
	10 days	224	71	27

Source: Adapted from Hill, 1969, Table 1, p. 332. Reprinted by permission of the publisher.

A significant portion of the fluoride associated with plants is not absorbed but is merely bound to the leaf or stem surface. The amount bound is highly variable and can be as high as 75% (Thomas and Alther, 1966). Exposure of plants to airborne cryolite increases the tissue fluoride concentration; however, a considerable portion of this can be removed by washing the tissue (up to 70% in tomato and gladiolus) (Ledbetter, Mavrodineanu, and Weiss, 1960; McCune et al., 1965). As usual, tissue content depends on the fluoride concentration and exposure time, but few fluoride symptoms occur with cryolite, even at relatively high tissue fluoride concentrations.

As might be expected, soybean plants accumulate considerably less fluoride when exposed to nighttime fumigation with hydrogen fluoride as compared with daytime fumigations, presumably because of stomatal closure (Poovaiah and Wiebe, 1973). Plants exposed to nighttime fumigations were only slightly injured. On the other hand, Adams, Hendrix, and Applegate (1957) found that with the same extent of injury the foliar fluoride concentration was less in nighttime-fumigated plants than in daytime-fumigated plants. However, with nighttime fumigations, longer exposures at equivalent fumigant concentrations were required to produce injury.

4.2.2 Translocation

The amount of fluoride translocation occurring in plants depends on the site of absorption. For example, root absorption of fluoride by mature navel orange trees led to large increases in the fluoride content of roots and a small but significant increase in leaf fluoride content (Brewer et al., 1959) (Table 4.3). Growth was depressed when trees were grown in solutions containing 25 ppm fluoride. The large amount of fluoride found in small roots was perhaps due to the formation of insoluble calcium fluoride in the soil and its adherence to the root surface.

Peach seedlings exposed to 10 and 25 ppm fluoride in nutrient solution accumulated fluoride in leaf tissue to 220 and 261 ppm, respectively, whereas control leaf tissue contained only 6 ppm fluoride (Leone et al., 1948). Exposure of Rutgers tomatoes to nutrient solutions containing 10 and 25 ppm fluoride resulted in the accumulation of 82 and 277 ppm fluoride, respectively, in leaf tissue. Thus fluoride supplied to the root can be translocated (presumably by the transpiration stream) to aerial portions of the plant.

However, exposure of the aerial portion of the plant to fluoride does not lead to increases in the fluoride content of roots. Fumigation of tomato plants with 0.048 and 0.47 ppm fluoride (4.5- and 3.5-hr exposures respectively), which elevated fluoride levels in leaves, did not increase the root fluoride content above control values (Brennan, Leone, and Daines, 1950). Thus no significant fluoride translocation from shoots to roots occurred. Continuous fumigation with about 1 $\mu g/m^3$ of fluoride did not produce injury symptoms in alfalfa, orchard grass, chard, and romain lettuce, and most of the fluoride was found in the leaves, again indicating little translocation to roots (Benedict, Ross, and Wade, 1964).

Leaf margins and tips often have higher fluoride concentrations than the rest of the leaf blade (Benedict, Ross, and Wade, 1964; Garrec, Plebin, and Lhoste, 1975; Hitchcock, Zimmerman, and Coe, 1962). Movement within the leaf probably occurs through the transpiration stream (Zimmerman and Hitchcock, 1956). Fluoride is taken up and distributed mainly to the tip when cut leaves are placed in aqueous fluoride solutions (Davison, Marsland, and Betts, 1974). When the plant is exposed to particulate fluorides, little translocation is observed. McCune et al. (1965) found that gladiolus leaves exposed to cryolite contained the same tissue concentration in all portions of the leaf; exposure to gaseous hydrogen fluoride resulted in accumulation of considerably more fluoride in the tip region. New leaves of potted elm and black locust that had been exposed to atmospheric fluorides during the winter contained higher fluoride levels than control leaves, suggesting mobilization and translocation of fluoride from bark and buds to the new leaves (Keller, 1974).

TABLE 4.3. FLUORIDE CONTENT AND FRESH WEIGHTS OF MATURE NAVEL ORANGE TREES GROWN FOR 18 MONTHS IN SOLUTION CULTURES WITH AND WITHOUT FLUORIDE PRESENT IN THE NUTRIENT SOLUTIONS

Tissue analyzed	Fluoride content (ppm)[a]		Fresh weight (kg)	
	25 ppm fluoride added[b]	No fluoride added[c]	25 ppm fluoride added	No fluoride added
Spring flush leaves, 1-year-old	34	5.5	3.49	6.18
Branches, whole cross sections			3.58	4.49
Large (over 1 in.)	5.5	2.0		
Intermediate (½-1 in.)	6.0	2.5		
Twigs	10.0	5.0		
Trunk			2.98	5.06
Old xylem	3.0	1.0		
New xylem	3.5	2.0		
Phloem and bark	8.3	4.0		
Whole cross section	7.0	3.0		
Roots			8.13	6.31
Large roots, whole	67	6		
Center wood, old xylem	9	13		
Intermediate wood, new xylem	13	4		
Epidermis	641	3		
Small roots	20,000	46		
Fruit				
Rind	1.70[d]	0.27[d]		
Pulp, juice	6.14[d]	0.09[d]		

[a] On an 80°C dry weight.
[b] Average of two trees.
[c] Average of four trees.
[d] Fresh weight.

Source: Adapted from Brewer et al., 1959, Table 2, p. 185, and Table 4, p. 187. Reprinted by permission of the publisher.

4.2.3 Cellular Metabolism of Fluoride

Fluoride ions are very reactive and can be expected to combine with various molecular species within cells. Fluoride is an inhibitor of various metalloenzymes (Hewitt and Nicholas, 1963); the mechanisms of inhibition are discussed more fully in Section 4.3.1.6.

The observation in the 1940s that fluoroacetate is the toxic ingredient in the South African plant *Dichapetalum cymosum* demonstrated that certain plants have the ability to synthesize covalently bonded carbon-fluorine compounds (Peters, 1972*a*). Approximately 36 species and varieties, found mainly in Africa, Australia, and Brazil, are now known to synthesize fluoroacetate. *Oxylobium parviflorum* and *Gastrolobium bilobum* have been reported to have fluoroacetate concentrations as high as 12,500 ppm. Monofluoroacetate has been shown to be the toxic principle in *Gastrolobium grandiflorum* (McEwan, 1964), *Acacia georginae* (Oelrichs and McEwan, 1961), and *Palicourea marcgravii* (de Oliveira, 1963), three plants extremely poisonous to livestock.

Analysis of many other species, however, has failed to detect fluoroacetate or other organofluorides. The presence of specific organofluorides in plant tissues can be established by the use of extraction and analytical techniques that can cope with interfering substances. Vickery, Vickery, and Ashu (1973) discussed the interference of iron and pigments with the detection of monofluoroacetate in *Dichapetalum heudelotti*, a species in which organofluorides had not previously been detected. Peters and Shorthouse (1964) expressed the opinion that synthesis of the carbon-fluorine bond is not a general property of all plants. Kakabadse et al. (1971) supported this statement with the observation that Darjeeling tea, which has a high fluorine content, contains only inorganic fluoride. Peters (1972*b*), however, later acknowledged that many plants possess trace amounts of fluoroacetate and fluorocitrate and commented that they had detected fluorocitrate in commercial tea (about 30 μg per gram tea) by gas chromatography. Cheng et al. (1968) observed that soybeans grown in nutrient solution with sodium fluoride or with hydrogen fluoride fumigation also synthesized fluoroacetate and fluorocitrate. These compounds were identified by paper chromatography. Lovelace, Miller, and Welkie (1968) found both fluoroacetate (179 μg per gram leaf dry weight) and fluorocitrate (896 μg per gram leaf dry weight) in forage crops (pasture mix of *Medicago sativa* and *Agropyron cristatum*) grown near a phosphate plant. Control area crops contained no detectable fluoroacetate or fluorocitrate. In the above studies, additional support for the occurrence of fluorocitrate was established by experiments demonstrating the inhibition of purified aconitase. Later work by Yu and Miller (1970) produced gas chromatographic evidence for fluoroacetate and fluorocitrate in *A. cristatum*, although these compounds were not detected in all samples. Miller (1972) could not detect any fluoroorganic acids in grass samples from several locations. Ward and Huskisson (1969) reported that lettuce could convert fluoroacetate to fluorocitrate, but later work (Ward, 1972) showed the amount converted to be quite small (about 2%). There are not enough data to conclude that all plants have the ability to synthesize fluoroorganic acids, but the results with soybean and forage plants suggest this possibility.

Organofluorides have been extensively studied in species of *Dichapetalum* and *A. georginae*. No organic or inorganic fluorides were detected in *D. cymosum* seeds, but the leaves contained fluoroacetic acid. However, *Dichapetalum toxicarium* seeds, which are toxic, contained ω-fluorooleic acid and another long chain fatty acid, but the leaves contained no fluorides (Peters and Hall, 1960). Ward et al. (1964) later found a number of fatty acids (e.g., ω-fluoropalmitic acid) in *D. toxicarium* seeds and suggested that longer chain ω-fluoro acids were produced by additions of malonyl coenzyme A to shorter chain ω-fluoro acids. Vickery and Vickery

(1972), however, reported that *D. toxicarium* plants contain both fluoroacetate (450 ppm in young leaves, 60 ppm in mature leaves, and 1100 ppm in leaves adnate to flowers) and inorganic fluoride (65 ppm in young leaves, 14 ppm in mature leaves, and 85 ppm in leaves adnate to flowers). Tap and lateral roots contained 23 and <1 ppm fluoroacetate and 30 and 38 ppm inorganic fluoride respectively. The high fluoride content probably was not related to fluoride pollution because fluoride concentrations in the soil and water were low, and other plants in the vicinity did not contain more than 2 ppm fluoride.

Hall (1974) exposed cultured *A. georginae* (in either quartz or soil) to solutions of sodium monofluoroacetate or ammonium fluoride. In all cases, most of the fluoride found in the roots and leaves was inorganic (Table 4.4). No biosynthesis of fluoroacetate was detected in the plants. Indeed, the plants apparently metabolized the added monofluoroacetate to inorganic fluoride, although the existence of microbial degradation prior to uptake was not experimentally tested. Thus while the toxic principle of *A. georginae* is fluoroacetate (Oelrichs and McEwan, 1961), in vivo formation of fluoroacetate is not easily induced in cultured plants. Preuss, Birkhahn, and Bergmann (1970), however, did detect fluoroacetate in tissue cultures from *A. georginae* stem sections.

Metabolism of ^{14}C-labeled fluoroacetate in *A. georginae*, peanut, castor bean, and pinto bean produced labeled carbon dioxide (indicating cleavage of the carbon-fluorine bond) and radioactivity in neutral lipids and water-soluble fractions (Preuss, Lemmens, and Weinstein, 1968). Preuss and Weinstein (1969) later showed that germinating peanut seeds could cleave the carbon-fluorine bond in fluoroacetate. No fluorine was detected in fatty acids, and only inorganic fluoride was detected in the seedlings. No information was found concerning enzyme systems in plants which cleave the carbon-fluorine bond.

Hall (1972) and Hall and Cain (1972) collected and studied a variety of toxic plants and the soils in which they grew. Organofluorides were detected in soils, perhaps mainly as fluoroacetate, but no relation to organic fluoride content in the plant could be demonstrated. Hall (1972) reported data on total and organic fluoride content in portions of toxic plants (Table 4.4) and concluded that there were wide variations in leaf fluoride content in all species, that high total fluoride content does not necessarily mean a high organic fluoride content, and that differences in distribution within the plant existed in members of the same species growing in Africa and Australia.

In plants that contain fluoroacetate as the toxic ingredient, little is known about the specific environmental or physiological conditions that determine the amounts or distribution of organofluorides formed. Similarly, there is little information on why fluoroacetate is not toxic to those plants possessing measurable amounts of it. Fluoroacetate and fluoroacetamide have also been used as systemic insecticides for plants. Injury to plants occurred at doses 50 to 100 times that required to kill *Aphis fabae* (David and Gardiner, as cited in Treble, Lamport, and Peters,

TABLE 4.4. DISTRIBUTION OF FLUORINE IN TROPICAL PLANTS

Species	Tissue	Fluorine (ppm in air-dried tissue) Total	Acid-labile	Organic soluble[a] Total	Organic soluble[a] Acid-labile
Acacia georginae (toxic)	Seed	4	4	<1	<1
	Leaf	42	19	14	2
	Root	11	5	6	1
A. georginae (experimentally cultivated)	Leaf	33	15	<1	<1
	Root	36	29	<1	<1
Dichapetalum cymosum	Leaf	111	6	99	2
	Stem	341	23	242	2
	Small root	162	48	92	4
	Large root	196	139	17	2
D. guineense	Seed	19	12	10	3
	Leaf	9	7	2	2
	Stem	13	4	2	1
	Root	38	16	2	2
D. mossambicense	Leaf	11	6	4	2
	Stem	72	70	2	1
D. stuhlmannii	Seed (1)	870	622	292	42
	Seed (2)	1470	1360	93	13
	Seed shell (endocarp)	223	218	8	3
	Leaf	142	55	50	3
	Thin stem	321	164	126	3
	Thick stem	265	73	144	1
	Root (without bark)	1916	1440	22	4
	Root bark	385	337	19	16
D. toxicarium	Seed	1980	192	1800	169
	Leaf	145	55	32	3
	Stem	75	59	16	1
	Root	127	33	66	12
	Seed shell (endocarp)	19	9	8	1
Gastrolobium bilobum	Seed	1292	38	1230	42
	Seed pod	163	10	128	4
	Leaf	175	9	160	4
	Stem	34	10	7	<1
	Root	15	2	9	3
G. callistachys	Seed	473	21	443	6
	Leaf	35	4	30	2
	Stem	27	1	18	1
	Root	12	2	9	1
G. calycinum	Seed	760	35	669	7
	Leaf	8	4	4	1
	Stem	77	6	51	3
	Root	19	4	16	3
G. grandiflorum	Seed	488	10	256	2
	Leaf	11	6	2	1
	Stem	15	4	4	1
G. microcarpum	Leaf	27	8	3	<1
	Root	35	35		
G. spinosum	Seed	65	1	7	3
G. villosum	Leaf	26	8	18	1

TABLE 4.4 (continued)

Species	Tissue	Fluorine (ppm in air-dried tissue)			
		Total	Acid-labile	Organic soluble[a]	
				Total	Acid-labile
Oxylobium parviflorum	Seed	1400	44	1090	3
	Calyx (flower head)	877	34	744	6
	Corolla (petals)	206	6	119	1
	Mature leaf	116	9	110	3
	Young leaves and stems	465	23	440	20
	Thin stem	67	2	59	1
	Small root	10	2	7	3
Palicourea marcgravii	Seed	1228	30	1136	15
	Fruit stalk	1216	52	1010	6
	Leaf	455	146	278	8
	Stem	118	11	117	1
	Root	41	10	31	4

[a] Defined as extractable in alkaline 90% propanol.

Source: Adapted from Hall, 1972, Table 5, p. 861. Reprinted by permission of the publisher.

1962). Trebel, Lamport, and Peters (1962) found that fluoroacetate inhibited the aconitate hydratase (aconitase) of *Acer pseudoplatanus* tissue culture cells to a much lesser extent than pig heart aconitate hydratase. Inhibition by fluoroacetate was largely reversible by the addition of isocitrate. Louw, de Villiers, and Grobbelaar (1970) also observed that the aconitase of *D. cymosum* and of *Parinarium capense* was less sensitive than pig heart aconitase to fluoroacetate. Since 50 m*M* fluoroacetate did not cause citrate to accumulate in leaf discs of *D. cymosum* but did cause accumulation in the disc of *P. capense*, they suggested that *D. cymosum* did not convert fluoroacetate to fluorocitrate while *P. capense* did.

4.2.4 Distribution

4.2.4.1 Concentration in Tissues — For plants growing in unpolluted areas, the concentration of fluoride in tissues is usually between 2 and 20 ppm (National Academy of Sciences, 1971). The exact concentration depends on many factors such as species, organ examined, age, and physiological condition. Table 4.5 is a compilation of fluoride concentrations found in selected crop plants and a few noncrop plants grown in unpolluted areas or used as controls in fumigation experiments. The concentration of fluoride in most of these plants is between 0.4 and 30 ppm.

There are few data on the fluoride content of noncrop plants growing in unpolluted areas. Most plants do not accumulate fluoride from soil; however, some members of the Theaceae family are accumulator species. Thus leaves of camellia and tea contain extremely high concentrations of fluoride (Brewer, 1966; Thomas and Alther, 1966).

TABLE 4.5. FLUORIDE CONCENTRATIONS IN SELECTED PLANTS[a]

Plant	Part	Fluoride concentration (ppm dry weight)	Comments	Reference
		Forage and feed plants		
Alfalfa	Tops	0.8-36.5 (3.6 mean)	Area free of industrial pollution	Suttie, cited in Israel, 1974[b]
		7-15	Controls	Zimmerman and Hitchcock, 1956
		10	Controls	Benedict, Ross, and Wade, 1964
Orchard grass		10	Controls	Benedict, Ross, and Wade, 1964
Red clover, timothy	Tops	10	Mixed planting	MacLean and Schneider, 1973
Clover		6-13	Controls	Gisiger, cited in Groth, 1975
Grass, hay		1-6		Garber, 1967
Lucerne		2-4		Garber, 1967
Beet	Roots	2.9		Garber, 1967
	Leaves	7		Garber, 1967
Corn	Cobs	1.6		Garber, 1967
	Stalk and leaves	2.2-6.3		Garber, 1967
Rutabaga		4-7		Garber, 1967
		Grains		
Wheat	Grain	1		Garber, 1967
	Straw	2.3-6.4		Garber, 1967
Rye	Grain	1.5		Garber, 1967
	Straw	1.2		Garber, 1967
Barley	Grain	1.7		Garber, 1967
	Straw	9.0		Garber, 1967
Oats	Grain	0.5		Garber, 1967
	Straw	6.8		Garber, 1967
Corn	Grain	0.15-0.38		Garber, 1967
Rice	Grain	0.76		Garber, 1967
		Root crops		
Potato	Tuber	1.5-3.0		Garber, 1967
	Leaves	9-30		Garber, 1967
	Peel	5.0-22.5		Garber, 1967
Sugar beet	Root	3.3-6.0		Garber, 1967
	Leaves	12		Garber, 1967
		Vegetables		
Endive	Leaves	51	Controls	Benedict, Ross, and Wade, 1964
Lettuce	Leaves	23	Controls	Benedict, Ross, and Wade, 1964
		4.4-11.3		Garber, 1967
Spinach		35	Controls	Benedict, Ross, and Wade, 1964
		1.3-28.3		Garber, 1967
Cabbage	Outer leaves	9.5		Garber, 1967
	Edible part	1.5		Garber, 1967
Cauliflower	Flowers	0.9		Garber, 1967
	Leaves	0.8		Garber, 1967
Cress		4.4		Garber, 1967
Parsley		9.0		Garber, 1967
Celery	Leaves	5.7		Garber, 1967
	Leaflets	10		Zimmerman and Hitchcock, 1956
	Stalks	2		Zimmerman and Hitchcock, 1956
	Heart	4		Garber, 1967

TABLE 4.5 (continued)

Plant	Part	Fluoride concentration (ppm dry weight)	Comments	Reference
Carrots	Roots	0.4-8.4		Garber, 1967
	Tops	40		Garber, 1967
Radishes	Roots	1		Garber, 1967
	Tops	2		Garber, 1967
Onions		3.0		Garber, 1967
Beans		3.2		Garber, 1967
	Tendergreen leaves	13.2	Controls	McCune, Weinstein, and Mancini, 1970
	Pinto leaves	13.1	Controls	McCune, Weinstein, and Mancini, 1970
Tomato	Leaves	8.0	Controls	McCune, Weinstein, and Mancini, 1970
	Leaves	8-13		Zimmerman and Hitchcock, 1956
	Stem	2		Zimmerman and Hitchcock, 1956
	Fruit	2		Zimmerman and Hitchcock, 1956
Red beets		2.8		Garber, 1967
		Noncrop plants		
Peach	Leaves	28	Unpolluted area	Drowley, Rayner, and Jephcott, 1963
Aesculus hippocastanea	Leaves	3.4		Garber, 1967
Sambucus nigra	Leaves	2.5		Garber, 1967
Acer sp.		2.0		Garber, 1967
Iris germanica		2.1		Garber, 1967
Taxus baccata		0.4		Garber, 1967
Pinus silvestris		0.7		Garber, 1967
Water hyacinth	Leaf Petiole	25 60	Growing naturally in water containing 1 ppm fluoride	Ras et al., cited in Groth, 1975

[a] Plants either growing wild not near obvious fluoride pollution sources or plants used as controls in fumigation experiments.

Table 4.4 lists the fluoride content of some toxic tropical plants. The indicated fluoride concentrations are relatively high. Since these plants were grown in soils low in fluoride, accumulation in these species is significant. No comments were made on the possibilities of fluoride air pollution in the areas of collection.

The National Academy of Sciences (1971) listed several conclusions about the relationship between environmental fluoride concentrations and plant fluoride concentrations: (1) Soil is the main source of fluoride in vegetation which is not exposed to air pollution. (2) There is not necessarily a direct relationship between soil fluoride concentration and plant fluoride concentration, but plants in acid soils generally have higher fluoride concentrations than plants in alkaline soils. (3) Industrial air pollution exposes plants to both particulate and gaseous fluorides which are taken up (or surface bound) by aerial portions of the plant. The amount of fluoride deposited on soil does not increase the soil content enough to significantly increase root absorption of fluoride (and thus concentration in the plant).

If it is assumed that plants are passive accumulators of fluoride, a useful relationship may be expressed by the equation $\Delta F = KCT$, where ΔF is the change in tissue fluoride content due to atmospheric fluoride exposure, K is the apparent accumulation coefficient, C is the concentration of fluoride in air, and T is the time of exposure (National Academy of Sciences, 1971). Complicating circumstances arise, however, in applying this equation. K is not constant and is usually reported only for gaseous hydrogen fluoride exposures. Moreover, particulate fluorides are common in the atmosphere and could affect the determination of K in field studies. In fact, there is a lack of field data relating plant fluoride levels to atmospheric fluoride levels (Israel, 1974*a*; National Academy of Sciences, 1971). Such information is needed, however, to assess fluoride movement via feeds to animals. With the use of limed filter papers to measure airborne fluorides, Israel (1974*a*) found an average K value of 3.8 for field crops of alfalfa and orchard grass. This is about two times the value found by other researchers for gaseous hydrogen fluoride exposures. Israel's overall conclusion was that "factors governing the accumulation of gaseous and particulate fluorides are not well enough understood at this time to allow a rational interpretation of the field results."

Only limited data exist on deposition velocities (ν_g) for gaseous fluorides. Israel (1974*a*) reported values of 16 and 31 ppm/sec for gaseous fumigation experiments with alfalfa and field exposures with alfalfa and orchard grass. The corresponding velocities related to the leaf surface area (ν_1) were 3.2 and 6.2 ppm/sec.

In general, plants exposed to high levels of atmospheric fluorides have elevated fluoride levels. Table 4.6 gives the range of fluoride concentrations found in organs of different species exposed to varying concentrations of fluorides and the range of concentrations in those tissues which show toxicity symptoms. Fluoride accumulates primarily in leaves of fumigated plants, while fruits and seeds generally accumulate little fluoride (Pack and Sulzbach, 1976). Oat seeds may be an exception. There are many other examples of increased fluoride contents in plants sprayed or fumigated with fluorides. Additional data presented in greater detail are given in Figures 4.2 and 4.3 and Table 4.7.

The experiments cited used relatively high fluoride concentrations, but the data of Pack (1971*b*) showed that even with lower fumigation concentrations, bean leaves accumulated fluoride in amounts roughly proportional to the concentration of fumigating gas. For instance, tendergreen beans accumulated 260, 700, and 1200 ppm fluoride when exposed to fumigation concentrations of 2.2, 6.6, and 13.9 μg of fluoride per cubic meter respectively. Stem, petioles, and fruit also showed proportional increases with increased fumigant concentration; the absolute amounts were considerably smaller, however. In controlled exposures, the fluorine content of grass was found to be proportional to the fluoride concentration in air

TABLE 4.6. FLUORIDE VALUES IN TISSUES OF VARIOUS PLANTS

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
Alfalfa (*Medicago sativa*)	Exposed to 1.5 ppb F[c]	Leaves	Not specified		25.00-149.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		132.00-203.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		182.00	
	Field	Leaves	From industrial and nonindustrial areas (washed)	3.00-9.00	13.00-52.00	
	Field	Leaves	From industrial and nonindustrial areas (unwashed)	5.00-15.00	19.00-65.00	
Apple (*Malus* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		35.00-18.00	72.00-234.00
	Exposed to 5.0 ppb F	Leaves	Not specified		37.00	79.00-259.00
	Exposed to 10.0 ppb F	Leaves	Not specified			142.00-194.00
	Before and after exposure to HF gas	Leaves	Not specified	24.00	56.00	
Apricot (*Prunus armeniaca*)	Orchard, near Al reduction plant	Leaves	Not specified			247.00-403.00
	Exposed to 1.5 ppb F	Leaves	Not specified			58.00-130.00
	Exposed to 5.0 ppb F	Leaves	Not specified			83.00-84.00
	Exposed to 10.0 ppb F	Leaves	Not specified			107.00
	Field	Leaves	From industrial and nonindustrial areas (washed)	1.00-6.00	15.00-30.00	32.00-640.00
	Field	Fruit	From industrial and nonindustrial areas (washed)	0.04-0.25		1.35
	Field near steel plant	Leaves	Mature (washed)			168.00
	Field near steel plant	Leaves	Mature (unwashed)			336.00
Avocado (*Persea americana*)	Exposed to 5.0 ppb F as HF, 5 to 17 weeks	Leaves	Washed	5.00-17.00		195.00-1027.00
Bean (*Phaseolus* spp.)	Grown in soil to which NaF was added	Leaves	Not specified	19.00		<310.00
Blueberry (*Vaccinium* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified			34.00-53.00
	Exposed to 5.0 ppb F	Leaves	Not specified			72.00-103.00
	Exposed to 10.0 ppb F	Leaves	Not specified			64.00

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
Buckwheat (*Fagopyrum* spp.)	Soil with and without F added (limed)	Leaves	Not specified	10.00	87.00-900.00	2450.00-9900.00
	Soil with and without F added (unlimed)	Leaves	Not specified	59.00		
	Grown in soil to which NaF was added	Leaves	Not specified	16.00-62.00	101.00	533.00-1910.00
	Grown in acid soil; exposed to HF gas	Leaves	Not specified	37.00-98.00		594.00-1388.00
	Grown in limed soil to which HF was added	Leaves	Not specified	10.00	900.00	
Carrot (*Daucus carota sativa*)	Exposed to 1.5 ppb F	Leaves	Not specified			250.00-323.00
	Exposed to 5.0 ppb F	Leaves	Not specified			309.00-723.00
	Exposed to 10.0 ppb F	Leaves	Not specified			307.00
Cherry (*Prunus cerasus*)	Before and after exposure to HF gas	Leaves	Not specified	20.00		112.00
	Exposed to 1.5 ppb F	Leaves	Not specified			51.00-62.00
	Exposed to 5.0 ppb F	Leaves	Not specified			51.00-53.00
	Exposed to 10.0 ppb F	Leaves	Not specified			126.00
Grapefruit (*Citrus paradisi*)	Exposed to 2.0 to 5.0 ppb F as HF for 6 months	Leaves	Washed	2.00-8.00	24.00-48.00	163.00-365.00
Lemon (*Citrus limon*)	Exposed to 2.0 to 5.0 ppb F as HF for 6 months	Leaves	Washed	5.00-9.00	16.00-24.00	72.00-146.00
Mandarin (*Citrus reticulata*)	Exposed to 2.0 to 5.0 ppb F as HF for 6 months	Leaves	Washed	3.00-6.00	40.00-59.00	85.00-345.00
Orange (*Citrus sinensis*)	Exposed to 2.0 to 5.0 ppb F as HF for 6 months	Leaves	Washed	8.00-11.00	20.00-116.00	158.00-334.00
	Field	Leaves	From industrial and nonindustrial areas (washed)	1.00-6.00	7.00-211.00	
	Cultures with about 1.0 and 25.0 ppm F	Leaves	Washed	2.00-5.50	34.00-60.00	

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
	Cultures with about 1.0 and 25.0 ppm F	Wood	Washed	1.00-5.00	3.00-10.00	
	Cultures with about 1.0 and 25.0 ppm F	Feeder roots	Washed	3.00-46.00	9.00-20,000.00	
Corn (*Zea maye*)	Before and after exposure to HF gas	Leaves	Not specified	8.00		302.00
	Exposed to 1.5 ppb F	Leaves	Not specified		151.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		99.00-133.00	178.00
	Exposed to 10.0 ppb F	Leaves	Not specified		85.00	147.00
Sweet corn	Exposed to 0.0 and 2.0 to 3.0 ppb F as HF in atmosphere	Leaves	Mature (washed)	5.00-14.00	29.00-67.00	48.00-491.00
	Exposed to 0.0 and 2.0 to 3.0 ppb F as HF in atmosphere	Husks	Washed	9.00	12.00	
	Exposed to 0.0 and 2.0 to 3.0 ppb F as HF in atmosphere	Kernels	Unwashed	2.00	10.00	
	Exposed to 0.0 and 2.0 to 3.0 ppb F as HF in atmosphere	Cobs	Unwashed	3.00	3.00	
	Exposed to 0.0 and 2.0 to 3.0 ppb F as HF in atmosphere	Stalks	Washed	6.00	15.00	
Cotton (*Gossypium* spp.)	Grown in neutral soil; exposed to HF gas	Leaves	Unwashed		5000.00	
Douglas fir (*Pseudotsuga taxifolia*)	Exposed to 1.5 ppb F	Needles	Not specified		102.00-238.00	
	Exposed to 5.0 ppb F	Needles	Not specified		103.00-200.00	
	Exposed to 10.0 ppb F	Needles	Not specified		212.00	
Elm, English (*Ulmus procera*)	Exposed to 1.5 ppb F	Leaves	Not specified			18.00-265.00
	Exposed to 5.0 ppb F	Leaves	Not specified			72.00
	Exposed to 10.0 ppb F	Leaves	Not specified			160.00
Fig (*Ficus* spp.)	Orchard near Al reduction plant	Leaves	Not specified			247.00-403.00

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
Fir (*Abies grandis*)	Exposed to 1.5 ppb F	Needles	Not specified		43.00–71.00	
	Exposed to 5.0 ppb F	Needles	Not specified		41.00–155.00	
	Exposed to 10.0 ppb F	Needles	Not specified		140.00	
Gladiolus (*Gladiolus* spp.)	Near fluoride-emitting factory	Leaves	Mature (washed)			149.00–275.00
	Near fluoride-emitting factory	Leaves	Mature (unwashed)			279.00–284.00
	Exposed to HF gas	Leaves	Not specified	7.00–10.00		30.00–50.00
	Grown in soil to which NaF was added	Leaves	Not specified	7.00–10.00		20.00–141.00
	Exposed to 1.5 ppb F	Leaves	Not specified			37.00–59.00
	Exposed to 5.0 ppb F	Leaves	Not specified			44.00–46.00
	Exposed to 10.0 ppb F	Leaves	Not specified			57.00
	Exposed to 0.0 and 5.0 ppb F as HF for several months	Whole leaves	Washed	2.00–28.00		129.00–733.00
Grape (*Vitis* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		51.00–117.00	
	Exposed to 5.0 ppb F	Leaves	Not specified			84.00–138.00
	Exposed to 10.0 ppb F	Leaves	Not specified			122.00
Wine grapes	Field	Leaves	From industrial and nonindustrial areas (washed)	2.00–8.00	12.00–27.00	22.00–462.00
	Field near steel plant	Leaves	Unwashed			336.00
	Field near steel plant	Leaves	Washed			168.00
Hemlock (*Tsuga canadensis*)	Exposed to 5.0 ppb F	Needles	Not specified		133.00	
Kale or collard (*Brassica oleracea acephala*)	Soil (limed) with and without F added	Leaves	Not specified	3.00–3.20	18.00–45.00	
	Soil (unlimed) with and without F added	Leaves	Not specified	3.70–37.00	20.60–96.00	96.00–262.00
Larch (*Larix* spp.)	Exposed to 1.5 ppb F	Needles	Not specified			53.00–62.00
	Exposed to 5.0 ppb F	Needles	Not specified			73.00–147.00
	Exposed to 10.0 ppb F	Needles	Not specified			106.00

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
Lilac (*Syringa vulgaris*)	Exposed to 1.5 ppb F	Leaves	Not specified			123.00
	Exposed to 5.0 ppb F	Leaves	Not specified			216.00
	Exposed to 10.0 ppb F	Leaves	Not specified		123.00	
Locust, black (*Robinia pseudoacacia*)	Exposed to 1.5 ppb F	Leaves	Not specified		113.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		153.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		80.00	
Maple (*Acer* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		80.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		209.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		129.00	
Marigold (*Tagetes* spp.)	Grown in soil to which NaF was added	Leaves	Not specified		200.00-600.00	
Mountain laurel (*Kalmia latifolia*)	Exposed to 1.5 ppb F	Leaves	Not specified		34.00-35.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		21.00-42.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		58.00	
Mulberry (*Morus* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		129.00	122.00-273.00
	Exposed to 5.0 ppb F	Leaves	Not specified		128.00-144.00	213.00
Nasturtium (*Tropaeolum* spp.)	Grown in soil to which NaF was added	Leaves	Not specified		42.00-60.00	
Parsnip (*Pastinaca satira*)	Exposed to 1.5 ppb F	Leaves	Not specified		177.00-218.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		146.00-470.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		208.00	172.00
Peach (*Prunus persica*)	Before and after exposure to HF gas	Leaves	Not specified	6.00-16.00	30.00	77.00-390.00
	Exposed to HF gas	Leaves	Not specified	6.00-15.00		30.00-50.00
	Grown in soil to which NaF was added	Leaves	Not specified	6.00		220.00-1442.00
	Exposed to 1.5 ppb F	Leaves	Not specified			54.00-92.00
	Exposed to 5.0 ppb F	Leaves	Not specified			89.00-101.00
	Exposed to 10.0 ppb F	Leaves	Not specified			94.00
	Near Al reduction plant	Leaves	Not specified	2.00-8.00		44.00-112.00 (fruit was damaged but leaves were not)
	Near Al reduction plant	Fruit peel	Not specified	2.00-5.00		19.00-34.00

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
Pepper (*Capsicum* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified			136.00-449.00
	Exposed to 5.0 ppb F	Leaves	Not specified			203.00
	Exposed to 10.0 ppb F	Leaves	Not specified		154.00	244.0
Petunia (*Petunia* spp.)	Grown in soil to which NaF was added	Leaves	Not specified		200.00-600.00	
	Exposed to 1.5 ppb F	Leaves	Not specified		66.00-84.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		273.00-362.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		148.00	
Pine (*Pinus ponderosa* and other species)	Field	Needles	Young	2.00-3.00		129.00
	Field	Needles	1 year old	2.00-3.00		229.00-330.00
	Field	Needles	2 years old	3.00-4.00		462.00
	Exposed to 1.5 ppb F	Needles	Not specified		26.00	39.00
	Exposed to 5.0 ppb F	Needles	Not specified		32.00	55.00-73.00
	Exposed to 10.0 ppb F	Needles	Not specified		106.00	70.00
	Near Al reduction plant	Needles	Partially expanded			15.00-20.00
	Near Al reduction plant	Needles	Newly expanded			30.00-35.00
	Near Al reduction plant	Needles	Expanded 3 months			80.00
	Exposed to 1.5 ppb F	Needles	Not specified		19.00-46.00	83.00
	Exposed to 5.0 ppb F	Needles	Not specified			54.00-72.00
	Exposed to 10.0 ppb F	Needles	Not specified			80.00
Pine, eastern white (*Pinus strobus*)	Exposed to 1.5 ppb F	Needles	Not specified		70.00-136.00	
	Exposed to 5.0 ppb F	Needles	Not specified		138.00	73.00
	Exposed to 10.0 ppb F	Needles	Not specified		67.00	41.00
Potato (*Solanum tuberosum*)	Exposed to 0.0 and and 3.0 ppb as HF	Leaves	Washed	19.00-27.00	163.00-267.00	
	Exposed to 0.0 and and 3.0 ppb as HF	Tubers	Washed	4.00-17.00	10.00-34.00	
Prune (*Prunus domestica*)	Near fluoride-emitting factory	Leaves	Not specified	9.00-20.00	43.00	121.00-496.00
	Near Al plants and 90 miles away	Leaves	Not specified	6.00-15.00	25.00	30.00-1400.00
	Near Al plants and 90 miles away	Twigs	Not specified	0.70-1.00		2.20-17.00
	Exposed to 1.5 ppb F	Leaves	Not specified			42.00-64.00
	Exposed to 5.0 ppb F	Leaves	Not specified			60.00-90.00
	Exposed to 10.0 ppb F	Leaves	Not specified			107.00

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
Raspberry (*Rubus* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified			88.00-243.00
	Exposed to 5.0 ppb F	Leaves	Not specified		104.00-176.00	216.00
	Exposed to 10.0 ppb F	Leaves	Not specified			162.00
Rhododendron (*Rhododendron* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		44.00-55.00	
	Exposed to 5.0 ppb F	Leaves	Not specified			85.00
	Exposed to 10.0 ppb F	Leaves	Not specified		30.00	
Rose (*Rosa* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		80.00-120.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		64.00-244.00	
	Exposed to 10.0 ppb F	Leaves	Not specified		175.00	
	Exposed to 0.0 and 3.0 ppb F for 6 months	Leaves	Mature (washed)	4.00-8.00		118.00-323.00
	Exposed to 0.0 and 3.0 ppb F for 6 months	Branches	Mixed, young and old (washed)	3.00-5.00	24.00	
	Exposed to 0.0 and 3.0 ppb F for 6 months	Flowers	Mature (rinsed)	4.00	13.00	
Spanish moss (*Tillandsia usneoides*)	Exposed in areas where air was contaminated with F	Whole plant	Unwashed	22.00-27.00	32.00-2418.00	
Spinach (*Spinacia oleracea*)	Grown in soil to which NaF was added	Leaves	Not specified	49.00	200.00-600.00	803.00-857.00
Spruce, Engelmann (*Picea engelmanni*)	Exposed to 1.5 ppb F	Needles	Not specified		80.00	57.00
	Exposed to 5.0 ppb F	Needles	Not specified		31.00	210.00-245.00
	Exposed to 10.0 ppb F	Needles	Not specified		149.00	
Squash (*Cucurbita* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified		114.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		85.00	134.00
	Exposed to 10.0 ppb F	Leaves	Not specified		99.00	179.00
Sweet pea (*Lathyrus odoratus*)	Exposed to 1.5 ppb F	Leaves	Not specified			327.00
	Exposed to 5.0 ppb F	Leaves	Not specified		118.00-144.00	148.00
	Exposed to 10.0 ppb F	Leaves	Not specified			141.00
Sweet potato (*Ipomoea batatas*)	Exposed to 0.0 and 3.0 ppb F as HF in atmosphere	Leaves	Washed	8.00-25.00		

TABLE 4.6 (continued)

Plant	Type of culture	Tissue sampled	Sample description	Fluoride concentration in dry matter (ppm)[a]		
				Intermediate range[b]	High range	Showing toxicity symptoms
	Exposed to 0.0 and 3.0 ppb F as HF in atmosphere	Tubers	Washed	3.10	91.00-146.00	220.00-319.00
Tomato (*Lycopersicon esculentum*)	Before and after exposure to HF gas	Leaves	Not specified	28.00-54.00		289.00-780.00
	Before and after exposure to HF gas	Fruit	Not specified	6.00		
	Grown in soil to which NaF was added	Leaves	Not specified	10.00-14.00	26.00-82.00	277.00-2179.00
	Exposed to 1.5 ppb F	Leaves	Not specified		231.00-247.00	
	Exposed to 5.0 ppb F	Leaves	Not specified		123.00-291.00	278.00
	Exposed to 10.0 ppb F	Leaves	Not specified		171.00	207.00
	Exposed to 10.0 ppb F as HF in greenhouse	Leaves	New (washed)	3.00-25.00		
	Exposed to 10.0 ppb F as HF in greenhouse	Leaves	Old (washed)	5.00-36.00	241.00	294.00-1365.00
Willow (*Salix* spp.)	Exposed to 1.5 ppb F	Leaves	Not specified			65.00-144.00
	Exposed to 5.0 ppb F	Leaves	Not specified			98.00-271.00
	Exposed to 10.0 ppb F	Leaves	Not specified			270.00

[a] There were no values showing deficiency symptoms or low ranges.

[b] Data derived from fumigation experiments are values found when no fluorine was present in the air. Otherwise, data in this column are from culture controls or nonindustrial areas.

[c] Refers to concentration of fluorine as hydrogen fluoride introduced into greenhouse fumigation chambers.

Source: Adapted from Brewer, 1966, Table 1, pp. 184-194. Data collected from several sources. Reprinted by permission of the publisher.

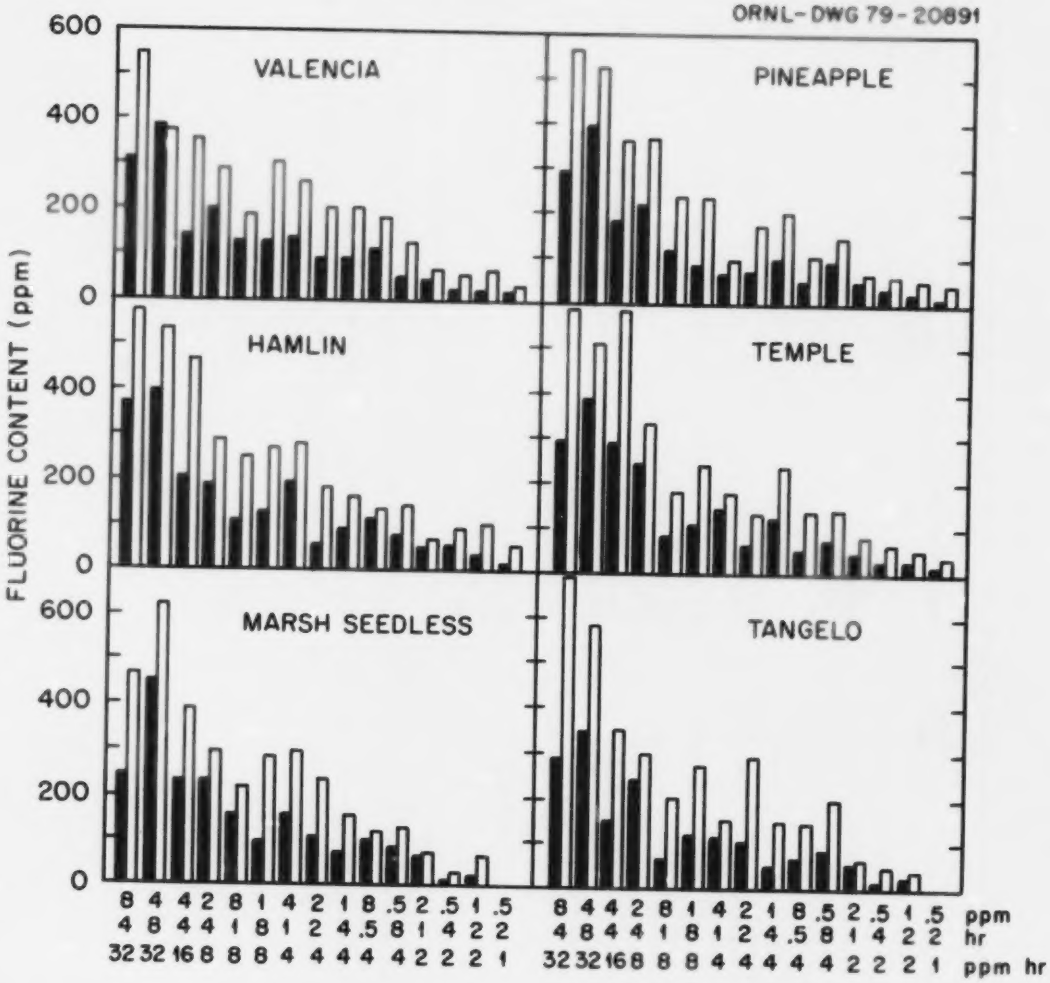

Figure 4.2. Fluorine content of washed (solid) and unwashed (shaded) leaves of six citrus varieties as affected by gaseous hydrogen fluoride exposure. Source: Adapted from MacLean et al., 1968, Figure 2, p. 448. Reprinted by permission of the publisher.

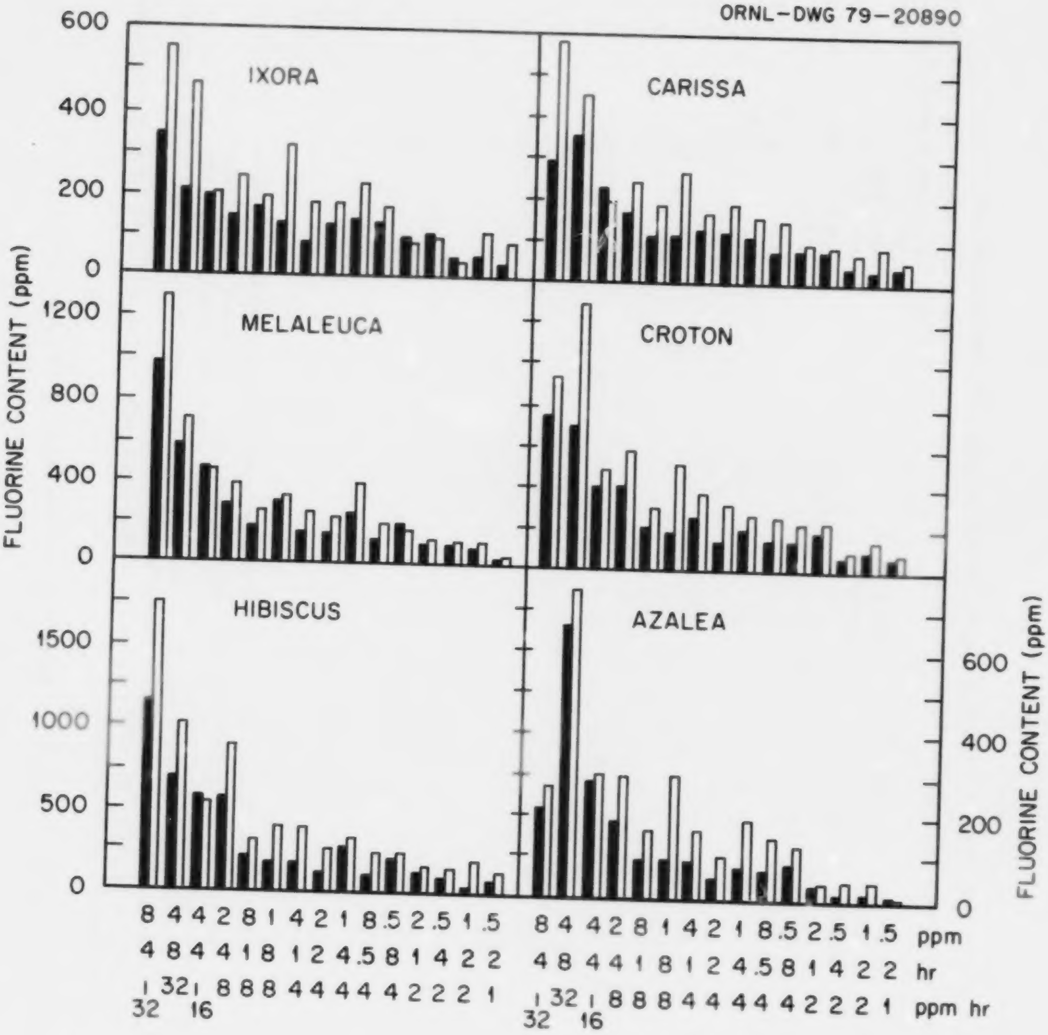

Figure 4.3. Fluorine content of washed (solid) and unwashed (shaded) leaves of six ornamental species as affected by gaseous hydrogen fluoride exposure. Source: Adapted from MacLean et al., 1968, Figure 3, p. 448. Reprinted by permission of the publisher.

TABLE 4.7. TREATMENTS AND TISSUE FLUORIDE CONCENTRATIONS FOR BEAN EXPERIMENTS

Bean variety[a]	Nutrient solution[b]	Day length (hr)	Days exposed to HF	Average HF concentration $\mu g\ F/m^3$	When exposed[c]	F content of plant tissue (ppm)					
						Leaflets		Stems, petioles		Fruit	
						Control	HF	Control	HF	Control	HF
RK	S	12	7	4.8	A	3.2	160[d]	0.3	2.4[d]	0.1	4.4[d]
RK	S	12	7	5.3	E	1.1	120[d]	0	2.0[d]	0.2	0.7[d]
RK	S	12	7	8.0	B	2.7	270	0.2	26	0	0
PPW	S	12	9	8.0	BE	1.2	180	0.6	12	0.3	0.6[d]
TC	S	12	14	7.8	BE	2.5	330	0.7	20	0.2	0.3[d]
FCL	L	12	7	4.5	BE	3.0	52[d]	2.0	3.3[d]	2.1	3.1[d]
TG	L	12	69	5.4	C	3.5	880	1.7	39	0.9	33
RK	L	11	70	2.2	C	1.0	260	0	8.3	0.7	4.2
TG	L	11	70	2.2	C	1.5	260	0	10	0.3	13
TG	L	11	70	2.2	C	1.0	340	0	24	0.5	21
PPW	L	11	70	2.2	C	0.3	220	0	19	0.3	21
RK	L	11	70	6.6	C	0.3	710	0	25	0	29
TG	L	11	70	6.6	C	0.3	700	0	29	0	46
TC	L	11	70	6.6	C	0	710	0	26	0	47
PPW	L	11	70	6.6	C	1.0	540	0	31	0	34
RK	L	11	70	13.9	C	3.6	1670	1.4	59	2.4	66
TG	L	11	70	13.9	C	2.4	1220	1.8	49	2.2	80
TG	S	13	82	10.5	C	4.5	690	1.5	28[d]	0[e]	13[d,e]
TG	L	14	92	9.1	C	4.5	775	0.8	30	0.5[e]	20[e]
TG	S	16	84	2.1	C	3.0	140	0.5	14	0[e]	14[e]
TG	S	10	92	0.60	C	4.0	54	1.5	3.0	0[e]	4.0[e]
TG	S	16	74	0.58	C	3.5	49	0.9	2.5	0[e]	3.6[e]

[a] RK — red kidney; PPW — pencil pod wax; TC — tendercrop; TG — tendergreen; FCL — fordhook concentrated lima.

[b] S — standard nutrient solution containing 200 ppm Ca; L — low Ca nutrient solution containing 40 ppm Ca.

[c] A — directly after flowering; E — during early flowering; B — directly before flowering; BE — beginning before flowering and extending into early flowering; C — continuously, from seeding to harvest.

[d] Samples for F analysis collected some time after HF treatment, when plants were harvested. All other F analyses of fumigated plants apply to samples collected at end of HF treatment.

[e] Mature seed only.

Source: Adapted from Pack, 1971*b*, Table I, p. 1129. Reprinted by permission of the publisher.

(Less et al., 1975). Gaseous hydrogen fluoride was more easily absorbed than particulate matter, and absorption was greater during wet cool weather (Figure 4.4).

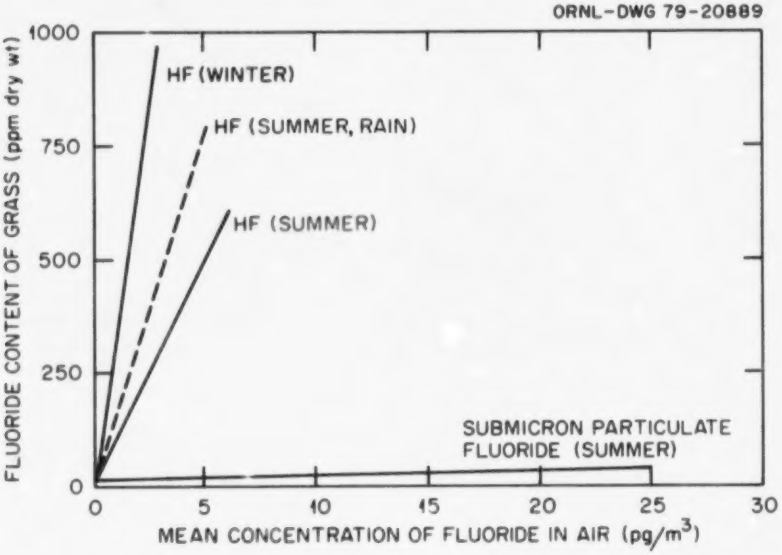

Figure 4.4. Comparison of uptake by grass of fluoride from hydrogen fluoride and from submicron particulate fluoride. Source: Adapted from Less et al., 1975, Figure 8, p. 159. Reprinted by permission of the publisher.

Continuous exposure to fluoride is more harmful to plants than intermittent exposure. For example, the intermittent spraying of citrus with either sodium fluoride or hydrogen fluoride was less injurious than continuous exposure to gaseous hydrogen fluoride. With intermittent exposure there is a chance for the absorbed fluoride to be "chemically fixed" between exposures, whereas there is no "rest period" with continuous exposure (Brewer, Sutherland, and Guillemet, 1969; Chang, 1975). Toxicity symptoms were the same with either treatment. Fluoride accumulation in foliage of timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) was greater with continuous exposure (10 days, 2.3 and 5.0 μg of fluoride per cubic meter) than with intermittent exposures (same concentrations, 2-day exposure to fluoride alternated with a 2-day exposure to filtered ambient air; total fluoride exposure time was 10 days) (Figure 4.5) (MacLean and Schneider, 1973). However, intermittent exposure yielded larger fluoride accumulation when total time was held constant and fumigant concentration was increased (MacLean, Schneider, and Weinstein, 1969). In all cases, plants were exposed to the same dose (defined as the product of fumigation concentration and the days of fumigation). The data illustrate the difficulty in predicting fluoride accumulation in field crops when only the

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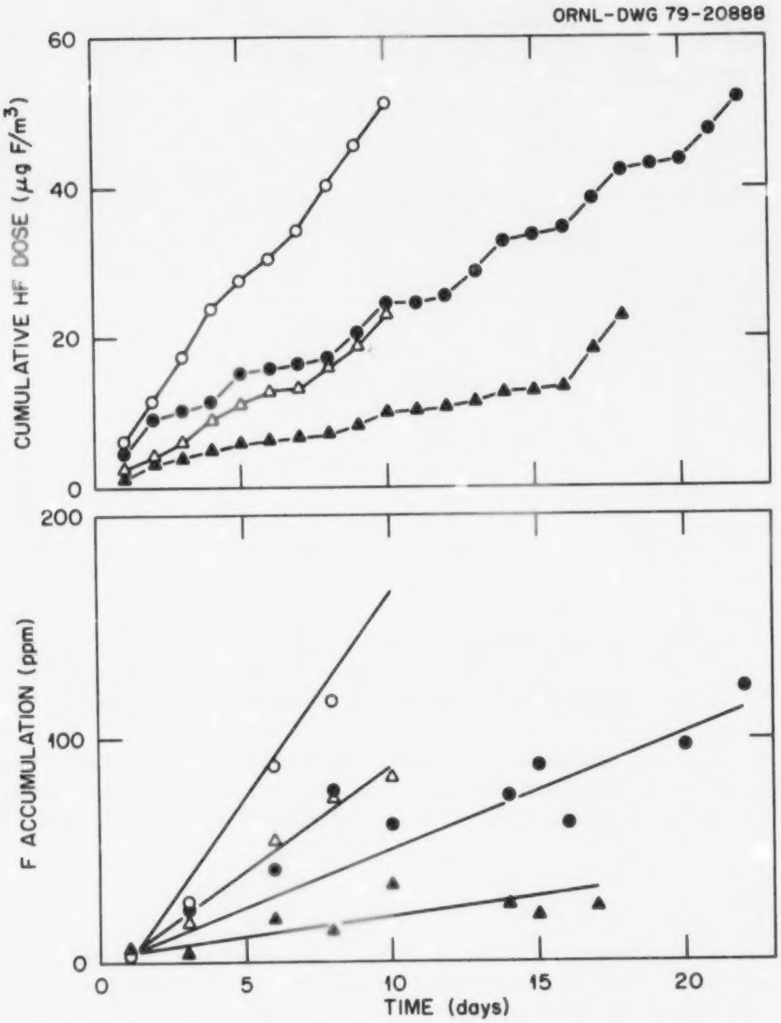

Figure 4.5. Fluoride accumulation in mixed planting of timothy and red clover with intermittent (solid symbols) and continuous (open symbols) fumigations. Source: Adapted from MacLean and Schneider, 1973, JOURNAL OF ENVIRONMENTAL QUALITY, Volume 2, page 501, by permission of the American Society of Agronomy, Crop Science Society of America and Soil Science Society of America.

average air concentration is known. In field situations, airborne fluoride concentrations and duration of exposure vary. Moreover, rain leaches fluoride from vegetation. Volatilization of organofluorides may also occur (Section 4.2.5). Exposure times and concentrations required to produce injury symptoms for several species are presented in Section 4.3.2.

4.2.4.2 Concentration in Plants Near Pollution Sources — Proximity to a fluoride pollution source usually increases the plant fluoride content.

Plants growing in the vicinity of a phosphorus extraction facility at Silverbow, Montana, contained much higher fluoride contents than control area plants (van Hook, 1974). Bluebunch wheatgrass contained average concentrations of 200 to 350 ppm fluoride in areas adjacent to the industry, while concentrations decreased to less than 50 ppm 1 to 2 miles away. Similarly, concentrations in juniper decreased from about 400 to 20 ppm fluoride. The rate of change in tissue fluoride concentrations with distance from the pollution source depended on the prevailing air currents.

Table 4.8 shows fluoride content of grasses, herbs, shrubs, and conifers found on plots surrounding the Anaconda Aluminum Company smelter in northwestern Montana (Carlson, 1973). Correlation of fluoride content with an index of injury implicated fluoride as the cause of injury. Fluoride concentrations in the lichen *Parmelia sulcata* decreased with distance from an aluminum factory; concentrations were about 900 ppm at 1 km, 870 ppm at 4 km, 500 ppm at 8 km, 340 ppm at 9 km, and 120 ppm at 15 km (LeBlanc, Rao, and Comeau, 1972). Fluoride levels increased significantly in Spanish moss placed in areas of known fluoride air pollution. Fluoride concentrations increased with exposure time and were indicative of the proximity of fluoride sources. The highest concentration detected was 2418 ppm, an increase from an initial concentration of 27 ppm. However, fluoride levels in the moss fluctuated with rainfall. A collection from the site that contained 2418 ppm was decreased to 1760 ppm after a rainfall of 2.89 in. (MacIntire, Hardin, and Hester, 1952). Ivos et al. (1970) reported high fluoride concentrations in plants near aluminum factories in Lozovac and Razine, Yugoslavia. Pine needles contained as much as 1806 ppm fluoride and "dusty" oleander leaves, 2747 ppm fluoride.

4.2.4.3 Subcellular Distribution — The subcellular distribution of fluorine-18 and fluorine-19 in tomato leaves after long and short fumigation exposures is presented in Table 4.9. Cell walls, chloroplasts, and soluble proteins contained the largest quantities of label. Chang and Thompson (1965) obtained similar results in cellular fractions from fumigated citrus leaves; however, because of cross contamination in organelle separation and preparation, they reasoned that accumulation was greatest in the chloroplasts, less in the cell-wall-nuclei fraction, and least in the mitochondria fraction. Some 60% of the fluoride was in the supernatant, rather than the organelle, fraction.

4.2.5 Bioelimination

There are few data on fluoride elimination from plant materials. Evidence for volatile organofluorides in homogenates of several plant species has been presented, but whether any loss of volatile fluorides occurs in vivo is unknown (Peters and Shorthouse, 1967). Monofluoroacetone was identified as the volatile fluoride lost from homogenates of *A. georginae*, but since it accounted for only about 13% of the total fluoride lost, other volatile compounds must exist (Peters and Shorthouse, 1971). Washing leaves of plants with distilled water can remove significant amounts of fluoride from plants previously exposed to either atmospheric fluoride or soil fluoride (Section 4.2.1.2) (Jacobsen et al., 1966). In the former case, the water probably removes airborne pollutants that

TABLE 4.8. CONCENTRATIONS OF FLUORIDE IN PLANTS NEAR THE ANACONDA ALUMINUM COMPANY SMELTER IN NORTHWEST MONTANA

Plot No.[a]	Average fluoride content (ppm dry wt)					
	Shrubs	Conifers		Herbs	Grasses	Grand average
		1969	1970			
Control No. 1-6	4.77-11.4[b]	3.5-10	3-11	5-12	1.3-16	4.79-10.36
R1-P1-7	3-108.5	5.8-300	9-40.8	12.5-188	2.5-70	6.8-122.36
R2-P1-7	3.6-112.7	5.5-143.5	2.3-20	5.5-93.8	2.5-83.3	3.7-91.21
R3-P1-7	10-1166.6	7-637	8-229	3.3-875.5	2.1-775	8.2-1004.3
R4-P1-10	8-778	8.93-681.5	4-116.5	5.7-628	5.8-234	6.88-604.14
R5-P1-10	11.05-1719	10.1-341	4.1-68.6	8.28-1038	5.5-600	7.46-1181.5
R6-P1-10	7.5-1125.3	13.5-1950	6-33	11-431	24.5-581	11.75-877.6
R7-P1-7	4.8-1073	10-168	4.5-22.3	7-600	20.5-338	10.87-871.7
R8-P1-7	11.8-399.8	14.2-119.8	9.2-175	13.3-235	8-110	12.6-409.8
R9-P1-7	5.6-108.7	7.8-110	10-39.5	6.5-51.5	5-41	7.72-70.97
R10-P1-7	6.8-76.5	9.2-133	3.5-42.5	10-45	6.5-38.5	7.54-66.3

[a] R — radius; P — plot.

[b] Samples were on transects radiating from the smelter. These values represent the range of fluoride concentrations found in all plots on the radius. Lower values were associated with plots distal from the fluoride source.

Source: Adapted from Carlson, 1973, Table 1*a*, p. 131. Reprinted by permission of the publisher.

TABLE 4.9. DISTRIBUTION OF ^{19}F AND ^{18}F IN VARIOUS CELLULAR CONSTITUENTS OF TOMATO LEAVES TREATED THROUGH THE AERIAL PORTIONS OF THE PLANT

Cellular constituent	^{19}F at the end of 10-day fumigation (ppm dry wt)	^{18}F after 2-hr fumigation (counts min^{-1} mg^{-1} dry wt)	
		Nonwashed	Washed
Cell walls	1685	142	155
Chloroplasts	693	176	145
Mitochondria	99	78	34
Microsomes	21		
Soluble proteins	371	592	662
Supernatant		82[a]	37[a]

[a] The concentration of ^{18}F is expressed for this fraction in counts min^{-1} mg^{-1} of fresh leaf tissue.

Source: Adapted from Ledbetter, Mavrodineanu, and Weiss, 1960, Table V, p. 346. Reprinted by permission of the publisher.

have settled on the leaves and any surface-extruded fluorides. The latter case implies that some of the fluoride that has been translocated to the leaf may eventually be deposited on the surface of the leaf and, thus, easily removed by brief water washings. Therefore, rainfall may be expected to remove a portion of the fluoride from the crown portion of the plant.

Loss of fluoride in plants also occurs through the seasonal loss of leaves, twigs, and roots. Hitchcock et al. (1971) suggested that the post-fumigation loss of fluoride from alfalfa plants was due mainly to leaf abscission and "weathering." Growth dilution (biomass added after fumigation) also leads to a decrease in tissue concentration of fluoride, but it is not an elimination mechanism. Weinstein (1961) observed a significant decrease in fluoride content of stem and leaf tissue from bean and tomato during a postfumigation period. The reason for this decrease could not be explained, but it was not due to growth dilution.

4.3 EFFECTS

Fluorides affect plants through chemical interactions that occur once the plant has taken up a quantity of fluoride. These effects can occur at various levels of organization, and ultimately all effects should be explicable in molecular terms. Table 4.10 summarizes some of the effects of fluoride observed at different levels of organization.

Fluorinated analogues of a variety of natural constituents have been used as inhibitors in numerous biological and biochemical studies. For example, fluorophenylalanine is an analogue of the amino acid phenylalanine; incorporation of this analogue into enzymes often results in reduced enzymatic activity. Since few data exist on the natural occurrence of formation of these analogues when plants are exposed to fluorides, their

TABLE 4.10. NATURE OF FLUORIDE-INDUCED EFFECTS IN PLANTS AT DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

Cell	Tissue or organ	Organism	Ecosystem
Altered cell milieu?			
Effects on enzymes and metabolites	Decreased assimilation Altered respiration		Increased fluoride in ecosystem
Modification of cell organelles and metabolism	Altered growth and development	Modified growth	Increase in fluoride burden of animals
Pathway disruption	Chlorotic lesions	Reduced reproduction	Fluorosis in animals
Cellular modification			
Disruption and death of cell	Necrotic lesions	Decreased fitness for environment	Change in plant community
	Death or abscission of leaf	Death of plant	Desolation

Source: Adapted from Weinstein and McCune, 1971, Table 1, p. 411. Reprinted by permission of the publisher.

effects are not discussed in this section. An exception is fluorocitrate, which can be formed from fluoroacetate. Production of fluorocitrate and fluoroacetate is discussed in Section 4.2.3.

4.3.1 Metabolic Effects

4.3.1.1 Photosynthesis — Although inhibition of photosynthesis by fluoride is well documented (Bennett and Hill, 1974; Chang, 1975; Thomas and Alther, 1966), the mechanisms by which it occurs have not been unequivocally determined. Suggested mechanisms include enzymatic inhibitions, loss of subcellular organization, and granulation of chloroplasts. Photosynthesis in sensitive plants (e.g., gladiolus and apricot) can be inhibited by a few parts-per-billion fluoride, whereas more resistant plants may require several hundred parts-per-billion fluoride to attain similar results.

Chlorosis is a common effect of exposure to fluorides (Chang, 1975). Newman (as cited in Wallis et al., 1974) found that fluoride decreased the chlorophyll content of bush bean leaves and suggested that fluoride inhibits photosynthesis by affecting chlorophyll synthesis. Both fluoride and chloride at 0.1 m*M* to 10 m*M* inhibited the in vivo production of chlorophyll and pheophytin *a* from ^{14}C-δ-aminolevulinic acid (ALA) in tobacco leaf discs (Wallis et al., 1974). Fumigation with 12.4 ppb hydrogen fluoride for nine days slightly decreased chlorophyll *a* and chlorophyll *b* contents of tomato and bean leaves, although recovery occurred in the postfumigation period (Weinstein, 1961).

Potassium fluoride (35 m*M*) inhibited oxygen evolution (Hill reaction) at pH 4.8 to 5.7 in isolated chloroplasts from bush beans (Ballantyne, 1972). Inhibition was overcome by increasing the pH above 5.6 and injecting magnesium and potassium salts into a chloroplast preparation. Inhibition did not occur with monofluoroacetate. The threshold for inhibition was 2 m*M* fluoride. Other examples of the fluoride inhibition of the Hill reaction are given in Chang (1975).

Apparent photosynthesis in barley and alfalfa was inhibited about 5% with 2-hr hydrogen fluoride exposures at 40 ppb fluoride and about 40% at 200 ppb fluoride (Bennett and Hill, 1973). Below about 150 ppb no detectable tissue necrosis occurred. At 40 and 100 ppb fluoride, carbon dioxide uptake decreased over the 2-hr fumigation period; recovery occurred after the end of the fumigation period, and near normal rates were observed the next day. At equivalent concentrations, inhibition of photosynthesis by hydrogen fluoride was 2 times as effective as ozone or chlorine, 4 times as effective as sulfur dioxide, 25 times as effective as nitrogen dioxide, and 40 times as effective as nitric oxide. Photosynthesis in detached leaves of three pine and six hardwood species was inhibited significantly when placed in 1 m*M* to 10 m*M* sodium fluoride solutions for 24 hr (McLaughlin and Barnes, 1975). Table 4.11 shows the decrease in apparent photosynthesis observed after exposure of samples collected on the given dates. In general, pines were more sensitive than hardwoods, and new needles more sensitive than old. Inglis and Hill (1974) observed that a 24-hr exposure to 50 m*M* fluoride almost completely inhibited carbon dioxide fixation (photosynthesis) in three moss species. At 25 m*M* fluoride, inhibition was 1% in *Tortula muralis*, 72% in *Hypnum cupressiforme*, and 90% in *Bryum argenteum*.

TABLE 4.11. SEASONAL EFFECTS OF FLUORIDE (1 m*M* NaF) ON PHOTOSYNTHESIS AND RESPIRATION OF FOLIAGE FROM THREE SPECIES OF PINES AND SIX SPECIES OF HARDWOOD

Species	Change from control (%)[a]											
	Apparent photosynthesis[b]						Dark respiration[b]					
	Pines											
	28 May	9 June	23 June	7 July	21 July	7 Aug.	28 May	9 June	23 June	7 July	21 July	7 Aug.
Loblolly pine												
New needles	ND[c]	-67	-79	-85	-69	-45	+8	+8	-9	-9	0	+18
One-year needles	-44	-40	-30	-75	-50	-54	+20	+10	0	+38	+20	+55
White pine												
New needles	-86	-100	-85	-79	-71	-74	-27	-36	+30	+33	+100	+50
One-year needles	-100	-89	-55	-59	-52	-46	+21	+40	+43	+67	+109	+136
Shortleaf pine												
One-year needles	-80	-90	-33	-94	-57	-60	+41	+13	+75	+15	+67	+23

	Hardwoods									
	Apparent photosynthesis					Dark respiration				
	3 June	17 June	1 July	15 July	29 July	3 June	17 June	1 July	15 July	29 July
Red maple	+22	-85	-66	-47	-57	+14	-13	+43	+51	+44
Sycamore	-17	-18	-48	+1	-11	+19	+22	+2	+29	+16
Yellow poplar	-81	-30	-30	-38	-80	+21	+71	+49	+47	+110
Red gum	0	ND[c]	-12	-31	-22	+5	ND[c]	+28	+52	+53
Dogwood	-27	-16	-35	-7	-9	+5	+22	+15	+22	+5
Sourwood	-83	+29	-50	-3	-55	-6	+48	-34	+5	+10

[a] Samples were collected at the dates listed and immersed in fluoride solutions for 24 hr. Measurements were performed after this exposure.

Source: Adapted from McLaughlin and Barnes, 1975, Table 2, p. 95. Reprinted by permission of the publisher.

A summary of the study by Bennett and Hill (1974) of the inhibition of photosynthesis by several air pollutants is shown in Figure 4.6. The relative order of inhibition of equal concentrations of these pollutants for barley and oats is $HF > Cl_2 \simeq O_3 > SO_2 > NO_2 > NO$. Although hydrogen fluoride can also induce stomatal closure and thus restrict carbon dioxide entry, inhibition of biochemical processes within the leaf is thought to be the major inhibitory mode. Recovery from hydrogen fluoride exposure occurs but is slower than that from other pollutants. Hill (1969) reported that a 24-hr average concentration of 48 ppb caused a 50% reduction of photosynthesis in strawberries; complete recovery required three weeks.

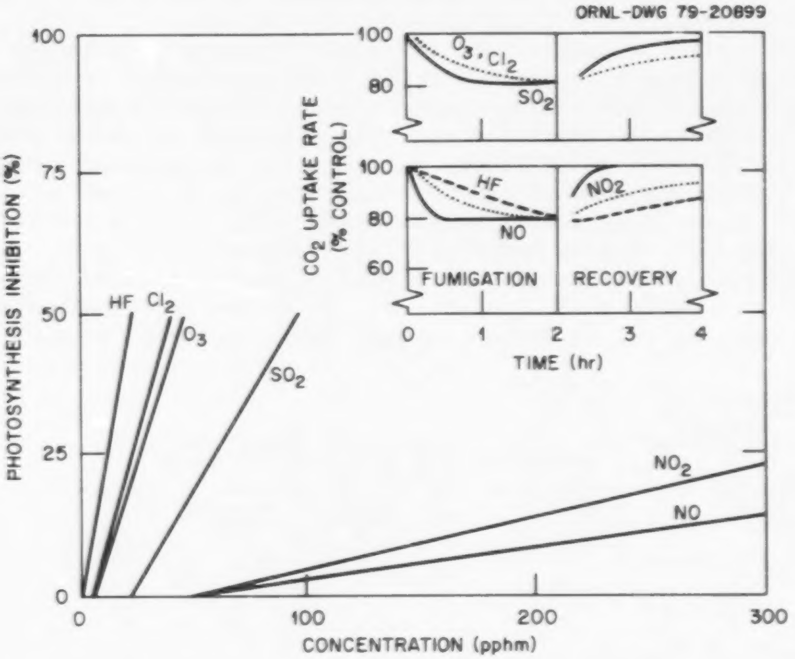

Figure 4.6. Inhibition of apparent photosynthetic rates of barley and oat canopies by 2-hr air pollution fumigations. Source: Adapted from Bennett and Hill, 1974, Figure 1, p. 118, in *Air Pollution Effects on Plant Growth*. Reprinted by permission of the publisher.

Although fluoride can inhibit photosynthesis in the field, it is difficult to determine if ambient fluoride levels are responsible for the observed damage. Ambient air pollution in the Los Angeles Basin reduced transpiration and apparent photosynthesis of lemon and navel orange trees. However, atmospheric levels of fluoride (averages of about 0.32 to 0.77 $\mu g/m^3$) were apparently not responsible, since controlled experiments designed to simulate ambient conditions failed to detect significant decreases in transpiration and photosynthesis (Thompson et al., 1967).

Inhibition of photosynthesis leading to yield depression without the formation of chlorosis or necrosis has been termed hidden injury and is discussed in Section 4.3.3.3.

4.3.1.2 Respiration and Oxidative Processes — Many reports provide evidence that exposure of plants to fluoride alters respiration (Chang, 1975). Generally, low concentrations stimulate respiration while higher concentrations inhibit respiration. The exact concentrations causing these effects depend on the species, exposure period, age of the plant material, and concentration of fluoride in the sensitive part of the cell (Weinstein, 1961). For example, exposure of detached leaves of three pine species and six hardwood species to 1 m*M* sodium fluoride increased respiration in most cases (McLaughlin and Barnes, 1975). However, the extent of stimulation varied considerably with species and with the age of the leaf (Table 4.11). Exposure of tomato and bean plants to fumigation with 1.6 ppb hydrogen fluoride increased respiration 67% and 72%, respectively, over control values (Weinstein, 1961). The data of Applegate, Adams, and Carriker (1960) (Table 4.12) showed that distinct inhibition of O_2 uptake by bush bean seedlings infiltrated with fluoride solutions occurred at sodium fluoride concentrations of 10 m*M* or greater. At the oldest developmental stage examined (five days "of germination"), 0.1 m*M* sodium fluoride stimulated respiration. Growing conditions (light or dark) and CO_2 concentration in the atmosphere in which O_2 uptake was measured affected the respiration rate. In the fluoride-sensitive plant *Chenopodium murale* 24- and 48-hr exposures of detached leaves to 1 m*M* KF decreased respiration, whereas in soybean, a fluoride-resistant species, similar exposure

TABLE 4.12. THE QO_2 RATIO (WATER INFILTRATED TO FLUORIDE INFILTRATED) OF BEAN SEEDLINGS AT THREE GROWTH STAGES

Fluoride concentration in infiltration solution (m*M*)	Light or dark germinated	CO_2 in Warburg flask (%)	QO_2 ratio[a]		
			Stage 1	Stage 2	Stage 3
100	Light	0	1.539	2.089	1.459
		0.6	1.784	3.188	3.406
	Dark	0	1.625	2.779	1.769
		0.6	2.420	3.428	2.214
10	Light	0	1.049	1.209	1.085
		0.6	1.254	1.921	1.912
	Dark	0	1.118	1.454	1.253
		0.6	1.287	1.910	1.299
1	Light	0	1.048	0.941	0.920
		0.6	1.168	1.327	0.994
	Dark	0	1.116	1.111	0.944
		0.6	1.199	1.469	1.007
0.1	Light	0	1.047	0.903	0.703
		0.6	1.164	0.918	0.789
	Dark	0	1.146	1.015	0.829
		0.6	1.196	1.122	0.818

[a] QO_2 — microliters of O_2 uptake per milligram dry weight.

Source: Adapted from Applegate, Adams, and Carriker, 1960, Table 2, p. 341. Reprinted by permission of the publisher.

slightly increased respiration (Yu and Miller, 1967). Except at one-day exposures, younger leaves showed more fluoride-induced respiration than older leaves. They also showed the greatest inhibition after four to five days of exposure. In general, respiration was not inhibited until visible necrotic injury was severe. In *Cetraria islandica* (lichen), exposure to 5 m*M* sodium fluoride increased respiration over a 5-hr exposure period, but respiration decreased after a 24-hr exposure to 70% of control (Vainshtein, 1973). Higher concentrations were inhibitory over the whole 24-hr exposure period.

Although the reason for fluoride-stimulated respiration is not known, observations in *Chlorella pyrenoidosa* indicated that stimulation may be related to the amount of undissociated hydrogen fluoride in the medium (McNulty and Lords, 1960). Higher concentrations of phosphorylated nucleotides occurred in fluoride-treated cells, and since respiration rate is thought to be governed by the ADP levels (or the ATP/ADP ratio), high ADP levels might stimulate respiration.

Dinitrophenol, an uncoupler of mitochondrial phosphorylation from electron transport, increased the rate of respiration in fluoride-treated soybean leaves to a lesser extent than in untreated tissues (Yu and Miller, 1967). This suggests that fluoride-induced respiration results from increased utilization of ATP, providing higher ADP pools in the cell. Heatherbell, Howard, and Wicken (1966) found that sodium fluoride slightly decreased oxygen consumption and phosphorylation in mitochondria isolated from etiolated pea seedlings.

Leaf tissue isolated from soybean plants fumigated for 48 hr with 9 to 12 $\mu g/m^3$ hydrogen fluoride showed increased respiration, mitochondrial succinate oxidation with ATPase activity, and decreased mitochondrial phosphorylation (Miller and Miller, 1974). After a 96-hr exposure, all the above activities were less than control values. Tightly coupled mitochondria, isolated from etiolated soybean hypocotyl and then exposed to concentrations of from 0 to 67 m*M* potassium fluoride, showed no increase in respiration when succinate, malate, or NADH was used as the substrate. Exposure of hypocotyl tissue itself to 10 m*M* potassium fluoride produced no stimulation in respiration. The osmotic properties of corn shoot mitochondria treated with potassium fluoride suggested that fluoride altered the permeability of the mitochondrial membranes. The authors concluded that fluoride may be acting at the membrane level, leading to an altered mitochondrial metabolism. The higher ATPase activity in fluoride-treated green tissues would increase ADP concentration, thus stimulating respiration. At longer periods of exposure to fluoride, higher internal concentrations of fluoride would occur and could, therefore, inhibit respiratory enzymes.

Miller and Miller (1974) discussed evidence that fluoride inhibits a variety of respiratory enzymes: succinic, malic, and NADH dehydrogenases; enolase; phosphoglucomutase; hexokinase; and ascorbic acid oxidase (Section 4.3.1.3). Levels of several enzymes were examined in soybeans fumigated with hydrogen fluoride. Glucose-6-phosphate dehydrogenase, cytochrome oxidase, peroxidase, and catalase activities increased in the fumigated leaves (Lee, Miller, and Welkie, 1966). Exposure of isolated enzymes to fluoride, however, showed glucose-6-phosphate dehydrogenase, cytochrome oxidase, and catalase to be unaffected while peroxidase was inhibited. There are no data on the turnover of these enzymes in fluoride-treated tissues. Peroxidase and cytochrome oxidase activities determined by histochemical procedures were highest in the phloem near the necrotic areas of *Pelargonium zonale* leaves fumigated with hydrogen fluoride (Poovaiah and Wiebe, 1971). This study provides in vivo evidence to support the above in vitro findings of Lee, Miller, and Welkie (1966). However, observed increased respiration is apparently not due to direct stimulation of enzymatic activity. The manner and site in which fluoride initiates injury are unknown.

4.3.1.3 Carbohydrate Metabolism — Fluoride is known to inhibit glucose catabolism in isolated plant tissues (National Academy of Sciences, 1971). The manner in which this occurs in vivo is unknown. In vitro, fluoride can inhibit enolase, the enzyme in the glycolysis pathway that converts D-2-phosphoglyceric acid to phosphoenolpyruvic acid (Lehniger, 1975; Miller, 1958). Inhibition occurs through the formation of a magnesium fluorophosphate complex.

The use of the pentose phosphate pathway would bypass inhibition of enolase and thus confer a selective advantage (increased fluoride resistance) to varieties using this pathway to a larger extent. Ross et al. (1968) examined this possibility by studying the release of labeled carbon dioxide from glucose containing ^{14}C in the 6 position (6-CO_2) and in the 1 position (1-CO_2). These studies were made on leaf discs from varieties of gladiolus that differed in their susceptibility to fluorides. Although there were considerable differences, varieties with increased resistance to fluoride generally had lower 6-CO_2/1-CO_2 ratios, suggesting that there might be a correlation between resistance and the increased use of the pentose phosphate pathway. Multiple correlation analysis showed that the 6-CO_2/1-CO_2 ratio accounted for 16% of the variation in leaf injury, flower color accounted for 32%, and leaf area for 15%.

While fluoride does inhibit growth, there is little proof that the growth inhibitory mechanism is through enolase, because other enzymes are also inhibited by fluoride. Ordin and co-workers demonstrated fluoride inhibition of the incorporation of ^{14}C glucose into cellulose and other components of the aqueous and acid-soluble cell wall fraction of oat coleoptile (Ordin and Skoe, 1963). From studies using glucose labeled with ^{14}C in several positions, they concluded that enolase was not inhibited in vivo.

In vitro, fluoride also inhibits phosphoglucomutase, the enzyme converting glucose-6-phosphate to glucose-1-phosphate (Yang and Miller, 1963*b*). Ordin and Altman (1965) demonstrated that oat coleoptile phosphoglucomutase was inhibited by fluoride in vitro and suggested that this was "the major cause of fluoride-induced inhibition of cellulose biosyntheses." Later, however, glucose-6-phosphate and glucose-1-phosphate pools were observed not to change with fluoride treatment (Gordon and Ordin, 1972). UDP-glucose pools did decrease after fluoride treatment. Thus inhibition in this system appears not to be related to phosphoglucomutase inhibition but to some other process that reduces incorporation of glucose into wall materials. Other researchers report that phosphoglucomutase is not inhibited by fluoride. De Moura, Le Tourneau, and Wiese (1973) found that potato tuber phosphoglucomutase was relatively insensitive to fluoride in vitro, and their preliminary data suggested that it also was not susceptible in vivo. Fluoride at 0.5 m*M* and 1.25 m*M* does not inhibit corn phosphoglucomutase in vitro, but at 0.5 m*M* and 1 m*M* it does inhibit corn seedling root growth 20% and 40% respectively (Chang, 1968). These studies provide further evidence that growth inhibition is not due to the in vivo inhibition of phosphoglucomutase.

Yang and Miller (1963*a*, 1963*b*) examined the metabolic effects of fluoride fumigation on soybean plants. After three- to five-day exposures to 0.03 ppm hydrogen fluoride, the fluoride content in the leaves was about 200 μg/g (on a fresh-weight basis). Fumigated leaves contained less sucrose and increased levels of reducing sugars and organic acids. Respiration increased 30% and 100% above controls (Yang and Miller, 1963*a*). Pipecolic acid accumulated in necrotic regions of the leaf. These data suggest that sucrose synthesis is inhibited by fluoride. The four known enzymes that convert glucose-6-phosphate to sucrose (phosphoglucomutase, UDP-glucose pyrophosphorylase, UDP-glucose-fructose transglucosylase, and UDP-glucose-fructose 6-phosphate transglucosylase) differ in vitro in their sensitivity to fluoride (Yang and Miller, 1963*b*). Soybean phosphoglucomutase is very sensitive to fluoride, while UDP-glucose-fructose transglucosylase is only slightly sensitive. UDP-glucose pyrophosphorylase is completely insensitive to fluoride. Increased dark carbon dioxide fixation occurs in fluoride-treated leaves and may explain the elevated levels of organic and amino acids found in necrotic leaves (Yang and Miller, 1963*c*).

The metabolism of phosphorus compounds is intimately related to carbohydrate metabolism. Pack and Wilson (1967) cited references showing that the enzymes enolase, phosphoglucomutase, phosphatases, and phosphorylases (all concerned with phosphorus metabolism) are inhibited by fluorides. They also showed, however, that levels of some 20 phosphorus metabolites in bean seedlings are not altered by exposure to 14 μg of hydrogen fluoride per cubic meter — exposure that produced no injury but produced 275 ppm fluoride in tissue. Thus, inhibition of enzymes concerned with acid-soluble phosphorus compounds did not occur in vivo.

McCune, Weinstein, and Mancini (1970) reported that there was "no consistent effect on the levels or composition of acid-soluble nucleotides" in fluoride-fumigated leaves of beans, tomatoes, and corn (4.8 to 10.7 μg of fluoride per cubic meter, 4 to 12 days). However, fumigated leaves did show reduced incorporation of phosphorus-32 into nucleotides, possibly because of decreased uptake of translocation of phosphorus.

Phytase from germinating pea seeds was inhibited 80% with 1 m*M* fluoride (Hauskrecht, 1972). Phytase activity in seeds increased with germination, presumably supplying phosphate for activation of various processes. Although conclusive experimental evidence is lacking, this may provide an alternative explanation for inhibition of germination by fluoride. Chang (1973) also observed that fluoride (0.1 m*M* to 10 m*M*) inhibited phytase activity in vitro (13% to 54% inhibition respectively).

4.3.1.4 Effects of Fluoride on Ribosomes with RNA Metabolism — The growth rate of corn seedling roots was decreased by treatment before germination with 0.01 *M* sodium fluoride for 1.5 to 7 hr. After exposure, the seeds were washed with deionized distilled water, treated lightly with a fungicide, then allowed to germinate and grow to a standard size of 12 ± 3 mm in a moist chamber containing deionized distilled water. Control seedling roots grew 0.36 mm/hr, whereas treated seedling roots grew only 0.28 mm/hr after 1.5-hr exposure, 0.24 mm/hr after 3-hr exposure, 0.20 mm/hr after 5-hr exposure, and 0.17 mm/hr after 7-hr exposure (Chang and Thompson, 1966). The RNA content of the 3 mm root tips was directly correlated with growth rates, but the DNA content was not. Since the RNA content per cell was correlated with the growth rate for different fluoride exposures, an effect of fluoride on RNA metabolism was postulated. Inhibition of both cell elongation and cell division occurred.

Fluoride-induced changes in acid-soluble nucleotides were observed in roots produced from fluoride-treated corn seeds (Chang, 1968). The triphosphate nucleotides increased the most. The base composition of RNA was also altered by fluoride treatment. Further studies showed that fluoride reduced the amounts of both free and bound ribosomes (Chang, 1970*a*, 1970*b*), and ribonuclease activity increased in plastids, mitochondria, the soluble fraction, and to the greatest extent in microsomes. A review of some of the biochemical changes in ribosomal metabolism and changes in cellular RNA content that occur during fluoride inhibition of root growth can be found in Chang (1973, 1975).

4.3.1.5 Fluorides and Calcium Nutrition — Some data suggest that fluoride injury is due to a calcium deficiency caused by precipitation of calcium as calcium fluoride. Since the calcium content of the cell is usually much larger than the fluoride concentration observed after exposure to fluoride pollutants, it is necessary to postulate that much of the calcium is present as chelates or as insoluble complexes and that the actual free ionic calcium content is quite small. Thus buildup of fluoride could precipitate the small concentration of ionic calcium required for normal metabolism.

Ramagopal, Welkie, and Miller (1969) observed that 5-min fluoride treatments of wheat seedling roots produced the same response as calcium deficiency and that potassium oxalate, sodium ethylenediaminetetraacetate, and sodium monofluoroacetate additions all elicited a similar response. Garrec et al. (1974) found that both fluoride and calcium accumulated in the injured tips of fir needles, presumably as calcium fluoride. However, although fluoride increased the total calcium level and the amount of calcium oxalate in these needles, other insoluble calcium salts (e.g., carbonates and phosphates) were only slightly increased. Brennan, Leone, and Daines (1950) reported that increased calcium in nutrient solutions led to increased fluoride content "in and about" tomato roots, apparently due to calcium fluoride precipitation.

In support of the idea that the mechanism of fluoride injury occurs through interference with calcium metabolism, Pack (1966) observed that injury to foliage from hydrogen fluoride fumigation was greatest when tomatoes were grown at the lowest calcium level (40 ppm calcium). At calcium levels of 40, 80, 120, and 200 ppm, hydrogen fluoride treatment caused significant decreases in total fruit weight per plant and in average fruit weight. A seedless condition of fruit was also more prevalent for hydrogen fluoride treatments at the 40 ppm calcium level. These effects were observed at the "high" hydrogen fluoride level of fumigation (6 μg of fluoride per cubic meter, 21 weeks), but not at the "low" level (2.9 μg of fluoride per cubic meter, 21 weeks); both levels are considerably higher than expected for outdoor areas. In other studies, the decreased sensitivity of mandarin leaves to atmospheric fluoride compared with gladiolus leaves, was attributed to the increased calcium and decreased silicon contents of mandarin leaves (Suketa and Yamamoto, 1975).

4.3.1.6 Mechanisms of Enzyme Inhibition — Fluoride inhibition of most enzymes probably occurs by complex formation with the polyvalent cations (iron, calcium, and magnesium) of metalloenzymes, making the ions unavailable for their role as cofactors. A discussion of the effects of fluoride on enzyme activity is given by Hewitt and Nicholas (1963). Enolase inhibition by fluoride occurs by the formation of a magnesium fluorophosphate complex that is inactive as a cofactor. A complex of fluoride, magnesium, and glucose-1-phosphate inhibits phosphoglucomutase activity. Several other enzymes requiring magnesium are also inhibited, to a varying extent, by fluoride. Similar examples exist for enzymes containing iron. The observed inhibition kinetics are often complex, and the extent of inhibition for different divalent metalloenzymes varies considerably. Most of these studies involve enzymes isolated from animal or microbial systems.

A variety of enzymes are inhibited in vitro by fluorides, but the relationship between the inhibition of specific enzymes and the physiological disorders and symptoms that occur is poorly understood (Section 4.3.1.3). For example, adenosine diphosphate sulfurylase, isolated from yeast or spinach leaves, is inhibited by fluorides (Burnell and Anderson, 1973). However, data are not available to determine if such inhibition occurs in vivo, and no information on aberrant sulfur metabolism in fluoride-treated plants was found.

4.3.2 Symptoms of Fluoride Accumulation

4.3.2.1 Foliar Symptoms — The best described symptoms of fluoride exposure relate to foliage. On monocotyledonous plants, marginal chlorosis and subsequent marginal necrosis often occur (Weinstein and McCune, 1971). On dicotyledonous plants, fluoride exposure results in leaf tip chlorosis, which develops downward along the margins with subsequent necrosis. In both types of plants, necrosis usually occurs without prior chlorosis and is prevalent in the tip and margin regions, areas where tissue fluoride concentrations are the highest (Treshow and Pack, 1970). Depending on the species, the necrotic zone may become yellow, brown, violet, or red. Interveinal chlorosis also occurs in some species, (maize, some citrus, and rose) exposed to low fluoride concentrations for a short period of time (Brewer, 1966).

There is wide variation in the susceptibility of different plant species (Table 4.13). Some ornamental and forest conifers are highly susceptible, while many broadleaf forest and ornamental deciduous plants are intermediate to resistant. Most important fruits and berries are at least moderately susceptible, and some (e.g., peaches and blueberries) are very susceptible to fluoride injury. There is disagreement on the degree of susceptibility of some species or varieties (National Academy of Sciences, 1971).

Common agricultural species used as indicators of fluoride pollution include gladiolus, wine grapes, apricots, and ponderosa pines (Brewer, 1966). Nettle-leaf goosefoot (*Chenopodium murale*), chickweed (*Stellaria media*), pigweed (*Amaranthus retroflexus*), and annual bluegrass (*Poa annua*) are weed species useful as indicators.

The concentration of fluoride in tissues showing toxic symptoms varies with species (Table 4.6) (Brewer, 1966). With some exceptions, little injury is expected in plants containing less than 20 ppm fluoride or plants exposed to less than 0.2 ppb fluoride in air (Hill, 1969). Injury is shown in many species containing tissue levels of 20 to 200 ppm fluoride and in species exposed to fumigation concentrations of 0.2 to 4 ppb fluoride. Some plants are not damaged at tissue fluoride concentrations greater than 500 ppm (National Academy of Sciences, 1971). Various factors determine the exact response occurring under field conditions; these include environmental factors (light, water, and temperature), pollutant form, and biological factors (species, developmental stage, and age).

Severity of injury is not necessarily related to the fluoride content of the tissue, although a certain minimum level of fluoride is required before visible injury occurs. Examination of many species shows that there is no relationship between the number of stomata per leaf and the susceptibility of the species to hydrogen fluoride (Zimmerman and Hitchcock, 1956). The leaf-fluoride content of different rose varieties exposed to 1 to 3 ppb hydrogen fluoride is not related to the severity of chlorosis or necrosis that develops (Brewer, Sutherland, and Guillemet, 1967). Extensive work on the effects of fluorides on gladiolus showed that more resistant varieties accumulate more fluoride in the leaves than susceptible varieties (Hitchcock et al., 1971; Hitchcock, Zimmerman, and Coe, 1962). For a given variety, a significant linear relationship exists between the extent of tip burn and the concentration of fluoride. In mixed plantings of the forage crops timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.), high continuous hydrogen fluoride treatment produces severe tip burn, marginal necrosis, chlorosis, and high tissue content of fluoride. Intermittent exposure to hydrogen fluoride results in high tissue levels of fluoride; however, tissue damage is not as extensive as with continuous exposure to the same concentration. This suggests that detoxification might occur during the nonfumigation period. Injury, therefore, is related to the manner of exposure and the concentrations experienced during the life cycle.

TABLE 4.13. SENSITIVITY OF SELECTED PLANTS TO FLUORIDE

Sensitive

- Apricot, Chinese and Royal
  - *Prunus armeniaca* L.
- Box elder
  - *Acer negundo* L.
- Blueberry
  - *Vaccinium* sp.
- Corn, sweet
  - *Zea mays* L.
- Fir, Douglas
  - *Pseudotsuga taxifolia* Brit.
- Gladiolus
  - *Gladiolus* sp.
- Grape, European
  - *Vitis vinifera* L.
- Grape, Oregon
  - *Mahonia repens* Don.
- Larch, western
  - *Larix occidentalis* Nutt.
- Peach (fruit)
  - *Prunus persica* Sieb. and Zucc.
- Pine, Eastern white, lodgepole, Scotch, Mugho
  - *Pinus strobus* L.,
  - *Pinus contorta* Dougl.,
  - *Pinus sylvestris* L.,
  - *Pinus mugho* Turra.
- Pine, ponderosa
  - *Pinus ponderosa* Laws.
- Plum, Bradshaw
  - *Prunus domestica* L.
- Prune, Italian
  - *Prunus domestica* L.
- Spruce, blue
  - *Picea pungens* Englm.
- Tulip
  - *Tulipa gesneriana* L.

Intermediate

- Apple, Delicious
  - *Malus sylvestris* Mill.
- Apricot, Moorpark and Tilton
  - *Prunus armeniaca* L.
- Arborvitae
  - *Thuia* sp.
- Ash, green
  - *Fraxinus pennsylvanica* var. *lanceolata* Borkh.
- Aspen, quaking
  - *Populus tremuloides* Michx.
- Aster
  - *Aster* sp.
- Barley (young plants)
  - *Hordeum vulgare* L.
- Cherry, Bing, Royal Ann
  - *Prunus avium* L.
- Cherry, choke
  - *Prunus virginiana* L.
- Chickweed
  - *Cerastium* sp.
- Clover, yellow
  - *Melilotus oflicinalis* Lam.
- Citrus (lemon, tangerine)
  - *Citrus* sp.
- Geranium
  - *Geranium* sp.
- Goldenrod
  - *Solidago* sp.
- Grape, Concord
  - *Vitis labrusca* L.
- Grapefruit (fruit)
  - *Citrus paradisi* Mact.
- Grass, crab
  - *Digitaria sanguinalis* L. Scop.
- Lamb's-quarters
  - *Chenopodium album* L.
- Lilac
  - *Syringa vulgaris* L.
- Linden, European
  - *Filia cordata* Mill.
- Maple, hedge
  - *Acer campestre* L.
- Maple, silver
  - *Acer saccharinum* L.
- Mulberry, red
  - *Morus rubra* L.
- Narcissus
  - *Narcissus* sp.
- Nettle-leaf goosefoot
  - *Chenopodium* sp.
- Orange
  - *Citrus sinensis* Osbeck
- Peony
  - *Paconia* sp.
- Poplar, Lombardy and Carolina
  - *Populus nigra* L. and *Populus eugenei* Simon-Louis
- Raspberry
  - *Rubus idacus* L.
- Rhododendron
  - *Rhododendron* sp.
- Rose
  - *Rosa odorata* Sweet
- Serviceberry
  - *Amelanchier alnifolia* Nutt
- Sorghum
  - *Sorghum vulgare* Pers.
- Spruce, white (young needles)
  - *Picea glanca* Moench, Voss.
- Sumac, smooth
  - *Rhus glabra* L.
- Sunflower
  - *Helianthus* sp.
- Violet
  - *Viola* sp.
- Walnut, black
  - *Juglans nigra* L.
- Walnut, English
  - *Juglans regia* L.
- Yew
  - *Taxus cuspidata* Sieb. and Zucc.

Resistant

- Ash, European mountain
  - *Sorbus aucuparia* L.
- Ash, Modesto
  - *Fraxinus velutina* Torr.
- Asparagus
  - *Asparagus* sp.
- Birch, cutleaf
  - *Betula pendula* var. *gracilis* Roth.
- Bridal wreath
  - *Spiraea prunifolia* Sieb. and Zucc.
- Burdock
  - *Arctium* sp.
- Cherry, flowering
  - *Prunus serrata* L.
- Cotton
  - *Gossypium hirsutum* L.
- Current
  - *Ribes* sp.
- Elderberry
  - *Sambucus* sp.
- Elm, American
  - *Ulmus americana* L.
- Juniper, most species
  - *Juniperus* sp.
- Linden, American
  - *Tilia americana* L.
- Pear
  - *Pyrus communis* L.
- Pigweed
  - *Amaranthus retroflexus* L.
- Plane tree
  - *Platanus* sp.
- Plum, flowering
  - *Prunus cerasifera* Enrh.
- Pyracantha
  - *Pyracantha* sp.
- Squash, summer
  - *Curcurbita pepo* L.
- Strawberry
  - *Fragaria* sp.
- Tomato
  - *Lycopersicon esculentum* Mill.
- Tree of heaven
  - *Ailanthus altissima* L.
- Virginia creeper
  - *Parthenocissus quinquefolia* Planch.
- Willow, several species
  - *Salix* sp.
- Wheat
  - *Triticum* sp.

Source: Adapted from Treshow and Pack, 1970, Table D-1, p. D-3. Reprinted by permission of the publisher.

Atmospheric fluorides and root-absorbed fluorides can produce the same symptoms (Brennan, Leone, and Daines, 1950). Brewer, Sutherland, and Guillemet (1969) reported that sprays of sodium fluoride and hydrogen fluoride produced toxic symptoms in citrus trees indistinguishable from those produced by fumigations with hydrogen fluoride gas. Detached leaves placed in fluoride solutions developed symptoms similar to those developed through exposure to gaseous fluorides (Davison, Marsland, and Betts, 1974). The percent of the leaf area injured was proportional to the fluoride concentration in the aqueous solution. The authors are currently evaluating this method at the University of Newcastle (England) as a technique to determine relative susceptibility among various species.

While specific symptoms produced by excessive fluoride exposure are quite similar in many species, they are not unique and can be induced in some species by disease or environmental stress (National Academy of Sciences, 1971). For example, moisture stress can cause necrosis in leaf margins, and manganese deficiency in peach, cherry, and citrus can cause marginal chlorosis and necrosis. Treshow and Pack (1970) listed other examples that mimic fluoride injury. It is difficult to prove that a particular injury is due to fluoride exposure, especially in the field. Adams (1963) discussed the problem of relating injury symptoms to causal agents and presented modifications of Koch's postulates (originally used to relate disease to a causal agent) as a guide for experiments designed to determine causes of injuries.

Fluorides affect the water balance of plants. Tomato, buckwheat, and peach wilt when treated with high concentrations of fluoride (50 to 400 ppm) in nutrient solutions (Leone et al., 1948; Prince et al., 1949). At moderate fluoride levels (10 to 50 ppm), tip and marginal scorching (necrosis) occurs. Citrus trees wilt when exposed to 100 ppm fluoride in nutrient solution (Brewer et al., 1959). High fumigation concentrations produce extensive abscission of leaves and young fruits of citrus plants (MacLean et al., 1968). Wilting of the succulent portions of new growth also occurs.

Poovaiah and Wiebe (1973) found that hydrogen fluoride fumigation greatly reduces transpiration in soybean, producing partial stomatal closure in 1 hr and complete closure within 4 hr. The transpiration rate was greatly reduced by fumigation, whereas leaf temperature increased. Halbwachs (1970) found that trees injured by fluoride exhibit a decrease in suction tension with increasing leaf or needle injury. The suction tension in trees with an uneven water balance declines abruptly in the upper regions of the crown. Garrec, Plebin, and Lhoste (1975) demonstrated that increased transpiration occurs in the region of corn leaves undergoing necrosis. This effect is not unexpected, however, since necrosis results in tissue disruption, including plasmolysis, leading to decreased ability of tissues to regulate water loss.

Water status can affect the response of the plant to hydrogen fluoride (Zimmerman and Hitchcock, 1956). Exposure to periodic wilting conditions allowed tomato and sweet potato plants to be more resistant to fluoride injury even though there was an ample water supply during the fumigation period. This result was not due to the amount of fluoride absorbed, since tomato and corn plants exposed to periodic wilting contained higher fluoride concentrations than the watered controls.

Fluoride-induced injury symptoms in plants are discussed further in a report on injury symptoms observed in ten common crop species (Pack and Sulzbach, 1976). Injury symptoms caused by other agents resembling fluoride injury are discussed in Treshow and Pack (1970).

4.3.2.2 Histological Observations — Microscopic observations of fluoride injury are rare, and of the few observations made, most are of tissue with extreme damage rather than tissue just beginning to show toxicity symptoms. Treshow, Anderson, and Harner (1967) summarized Solberg's data on the histological changes that occur in pine needles showing fluoride injury. Phloem and xylem parenchyma become enlarged and distorted, followed by granulation, vacuolation, and eventual collapse of the protoplasm. The advancing region of collapsing mesophyll next to the necrotic zone is only a few cells in thickness. Carlson (1973) observed that fluoride injury in pine needles caused hypertrophy in phloem and transfusion parenchyma which led to collapse of xylem and phloem. Nuclear enlargement and chlorophyll destruction also occurred. Pine needles from trees growing near fluoride pollution sources or from low-dose fumigation experiments showed hyperplasia and hypertrophy of phloem cells and resin duct occlusion (Stewart, Treshow, and Harner, 1973). However, several other environmental stresses can also produce similar histological responses.

In cotton (*Gossypium hirsutum*), fewer chloroplasts occur in mesophyll cells of fluoride-treated leaves; the effect is more pronounced in spongy than in palisade mesophyll cells (Timmermann, Applegate, and Engleman, 1970). Electron microscopy of *Rubus hispidus* explants cultured in the presence of fluoride showed elongation of mitochondrial cristae and increased density of the stroma (Pilet and Roland, 1972). The number of free ribosomes diminished, and the endoplasmic reticulum dilated causing formation of vesicles.

4.3.2.3 Fruit Disorders — Fluoride exposure affects fruit production in a number of species. Peaches exposed to atmospheric fluoride concentrations too low to produce foliar symptoms developed a soft "suture red spot" syndrome (Facteau and Wang, 1972; Treshow and Pack, 1970). The extent of fruit injury is influenced by calcium and boron nutrition. Lime sprays are effective in reducing the occurrence of soft suture (Thomas and Alther, 1966).

Exposure of strawberry plants to 0.55 to 10.4 μg of fluoride per cubic meter produced deformed fruit, decreased the average fruit weight, and reduced the proportion of flowers that set fruit (Pack, 1972). No effect was found on the number of flowers formed. The data support the author's contention that hydrogen fluoride affects fruiting in plants by interfering with fertilization or seed development.

Tendergreen beans showed no foliar injury or consistent effects on fruiting with continuous exposures (60 to 92 days) of 2.2 μg of hydrogen fluoride per cubic meter (Pack, 1971*b*). Chlorosis was observed with continuous exposures of 6.6 and 13.9 μg of fluoride per cubic meter. At 13.9 μg of fluoride per cubic meter, dry and wet weights of bean tops were not affected; however, the number of fruits per plant, number of seeds per fruit, and dry weight of fruit were all significantly decreased. Continuous exposure to 2.1 μg of fluoride per cubic meter did not produce foliage symptoms but did increase the amount of starch found in a seed. Beans grown in an atmosphere containing 2.1, 9.1, and 10.5 μg of fluoride per cubic meter produced F_1 progeny that were less vigorous than controls and had increased abnormalities of early trifoliate leaves (Pack, 1971*a*). These effects appear related to the decreased starch content per seed or decreased seed size. Fewer F_3 generation plants had abnormalities; consequently, the inheritability of such characteristics is in doubt.

Tomato plants exposed to high concentrations of hydrogen fluoride produce small partially or completely seedless fruit. Plants on low calcium budgets respond similarly. The combined effects of hydrogen fluoride and low calcium appear additive; thus, fluoride apparently functions by interfering with calcium metabolism. Supporting evidence is found in experiments concerning the effects of hydrogen fluoride and low calcium on germination of tomato and cucumber pollen (Sulzbach and Pack, 1972). With low calcium (.15 m*M*), 2.6 and 10.5 m*M* sodium fluoride inhibits tomato pollen germination, but no fluoride inhibition is observed with higher calcium concentrations. Similar observations occur with pollen from fumigated plants. However, cucumber pollen germination is not inhibited at concentrations of fluoride lower than 10.2 $\mu g/m^3$, regardless of calcium nutrition.

Fruit production decreased in citrus trees sprayed with aqueous sodium fluoride, but no significant effects on fruit quality occurred at doses designed to yield foliar fluoride concentrations of 75 and 150 ppm (Brewer, Sutherland, and Guillemet, 1969). Fluoride exposure also caused necrosis, decreased tree growth, and smaller fruit yield. Most reduction in yield probably resulted from fluoride exposure during spring bloom and growth flush (Leonard and Graves, 1970, 1972). Extensive injury decreased the fruit yield the next year even though the tree was not exposed to additional atmospheric fluorides.

Examination of the fruiting response of ten important crop species exposed to hydrogen fluoride in growth chambers showed that soybean was the most sensitive (no seeds produced with continuous exposure of 0.64 μg of fluoride per cubic meter) and cotton the least sensitive (no effects at 8.0 μg of fluoride per cubic meter) (Pack and Sulzbach, 1976). The order of decreasing sensitivity was soybean, bell pepper, sweet corn, cucumber, pea, grain, sorghum, oat, wheat, barley, and cotton. Common responses included fewer seeds per plant and smaller seed size. The authors suggested that reduced seed production was due to the inhibition of pollen germination or pollen tube growth.

Fewer data are available on fluoride injury to flowers (National Academy of Sciences, 1971). Fluoride may reduce the number of flower buds and size of the flower, change the flower color, and produce necrotic zones

(Ionescu, Serbanescu, and Pal, 1972). Treshow and Pack (1970) stated that "flower petals are rarely injured in the field." However, petunia and cyclamen flowers were cited as exceptions. Gladiolus flowers are resistant to fluoride, even though their leaves are extremely sensitive. Cut gladiolus flowers developed injury symptoms when exposed to about 1 ppm fluoride in reservoir water (Marousky and Woltz, 1971). Flower development was inhibited by hydrogen fluoride in pepper (4.5 $\mu g/m^3$) and corn (8.7 $\mu g/m^3$) (Pack and Sulzbach, 1976).

4.3.2.4 Germination — The effects of fluoride on seed germination are reviewed by Thomas and Alther (1966). Germination is inhibited at higher fluoride concentrations, although the extent of inhibition depends on species and exposure time. For example, a 72-hr exposure of lentil seeds to 1 m*M* and 10 m*M* sodium fluoride reduced germination to 75% and 5% of control respectively. In some field experiments, increased germination was observed after application of fluorides to the soil.

4.3.3 Effects of Fluoride on Growth and Productivity

4.3.3.1 Implications of Agriculture — Weinstein and McCune (1971) discussed the effects of fluoride on agriculture (Figure 4.7). They noted that effects on the quality of agricultural produce are easier to establish than effects on quantity and that fluoride damage not only affects the product directly but affects production costs as well. Even if product quality or quantity is not affected, increased fluoride content in plants

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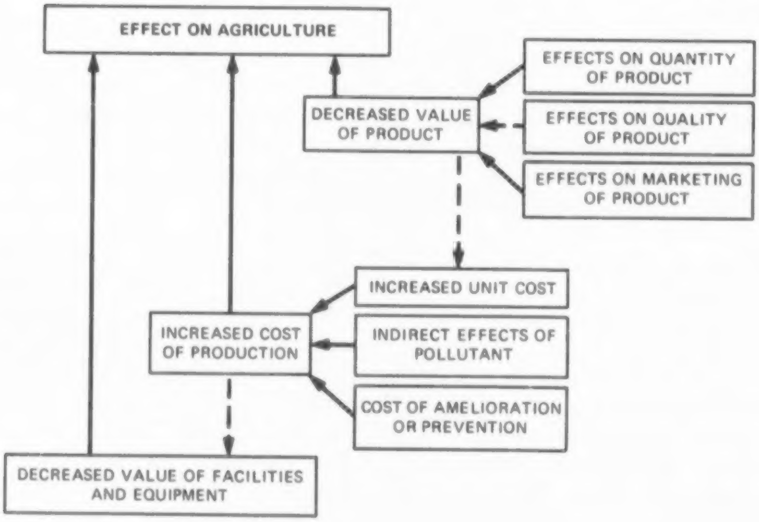
EFFECT ON AGRICULTURE
DECREASED VALUE OF PRODUCT
EFFECTS ON QUANTITY OF PRODUCT
EFFECTS ON QUALITY OF PRODUCT
EFFECTS ON MARKETING OF PRODUCT
INCREASED UNIT COST
INCREASED COST OF PRODUCTION
INDIRECT EFFECTS OF POLLUTANT
COST OF AMELIORATION OR PREVENTION
DECREASED VALUE OF FACILITIES AND EQUIPMENT

Figure 4.7. Possible effects of fluoride on agriculture. Source: Adapted from Weinstein and McCune, 1971, Figure 1, p. 412. Reprinted by permission of the publisher.

may lead to fluorosis in animals consuming these plants. These concerns are the impetus for studies relating atmospheric fluoride concentrations to tissue fluoride concentrations, growth inhibition, and visible damage symptoms — symptoms that might decrease the quality of the plant product or decrease plant productivity. Ultimately, such data could be used to recommend air quality criteria.

In establishing air quality standards for fluoride, three different criteria can be used: (1) the fluoride content of vegetation, (2) the fluoride content of the atmosphere, and (3) vegetation markings (necrosis, chlorosis, and other symptoms) (Hill, 1969). One should also consider that (1) fluoride is an accumulative toxicant, and injury is usually associated with long-term exposure; (2) gaseous and particulate fluorides differ in their phytotoxicity; (3) plant species and varieties differ greatly in susceptibility to fluoride; and (4) extremely low concentrations can cause damage to sensitive species (Hill, 1969). Hill discussed the advantages and problems of each approach and concluded that analysis for necrosis or chlorosis (damage assessment) is the best approach because this reflects the fluoride content in air. For a given area, species diversity, sensitivity, and air-concentration-time relationships are all manifest in such an assessment. However, Weinstein (1969) pointed out that examining the extent of fluoride damage is a useful procedure for evaluating and rectifying the proposed standard, not implementing it. He believes that more constant vigilance is necessary and that this could best be accomplished through "use of a series of provisional time concentration values for atmospheric fluorides which reasonably reflect the state of our present knowledge, in conjunction with appropriate atmospheric sampling stations, vegetation analysis, and periodic vegetation surveys." Obviously, air standards cannot be determined without adequate information relating air concentrations of fluoride to markings and fluoride accumulation in plants. By considering the factors listed by Hill (1969) and accumulating data concerning the relation of fluoride time-concentration exposure to injury (growth reduction and visible symptoms), a set of air quality criteria could be established for a given area with known species diversity. There is consensus that such data are lacking at the present time. Treshow and Pack (1970) noted the inability of investigators quantitatively to relate the degree of injury in plants to fluoride concentrations in plant tissue or in the air. Thus crop loss and injury can only be observed in the field (i.e., after the fact). They concluded that knowledge of vegetation fluoride levels is only useful to determine the effectiveness of air pollution control programs. Considerable disagreement thus exists concerning what criteria should be used to establish air quality standards. Furthermore, the adopted concepts should not be restricted to agricultural systems but should be extended to natural ecosystems as well.

Fluoride is an accumulative toxicant and injury in the field is usually caused by the accumulation of fluoride over a period of several weeks to several months. Unfortunately few long-term fumigation studies have been conducted to determine the relationship between long-term exposure and injury. Hill (1969) summarized his long-term exposure studies as follows: "In the greenhouse, continuous exposure to hydrogen fluoride in the concentration range 0.4 to 0.6 ppb for several months will cause severe injury to sensitive varieties of gladiolus, apricots, peaches, and corn."

To summarize relationships between atmospheric fluoride concentrations and injury, McCune (1969) presented log-log plots of the data from many literature reports. For each species, mean concentrations of fluoride in air are plotted on the ordinate and the duration of exposure is plotted on the abscissa; in addition, an indication is made at each point whether visible injury occurred, whether growth or yield was affected, and what tissue fluoride concentrations were found. Figures 4.8-4.11 give results for tomato, alfalfa, gladiolus, and sorghum respectively. Since the data were gathered from diverse literature sources, a number of factors contribute to the uncertainty of establishing permissible air concentrations of

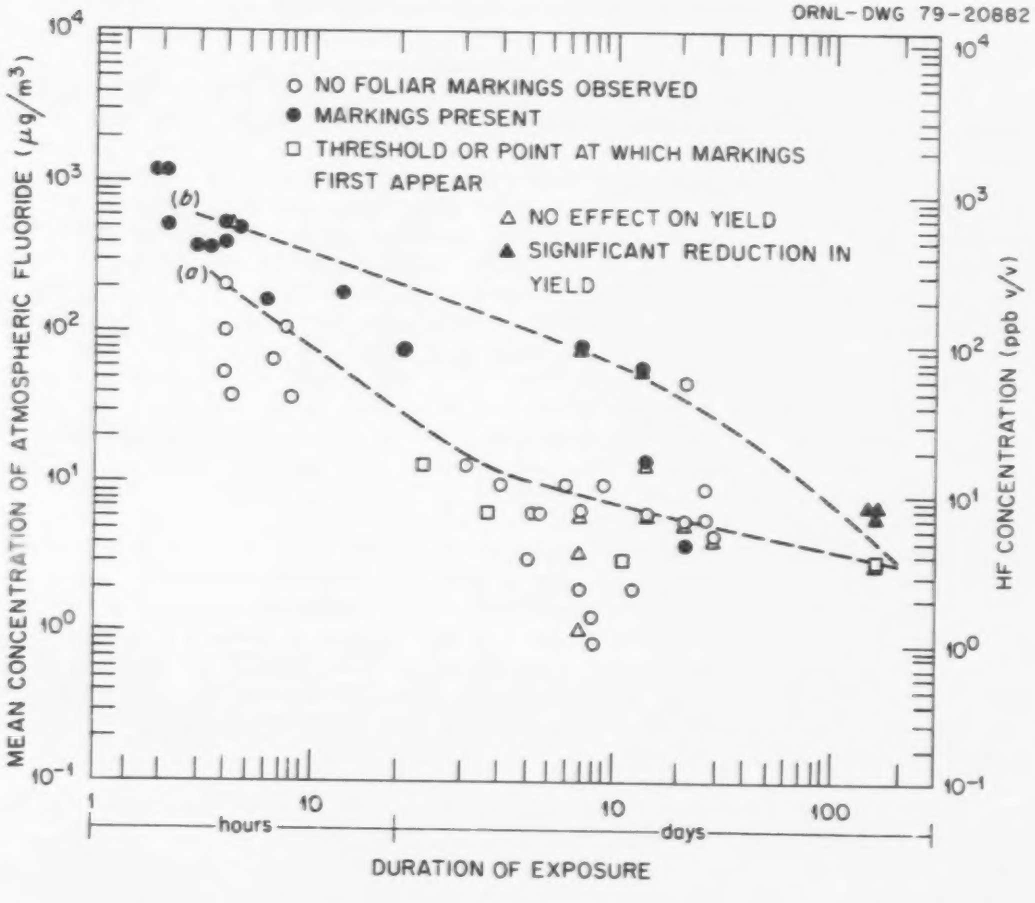

Figure 4.8. Relation of concentration and duration of exposure to effects of atmospheric fluoride on tomato. Adapted from McCune, 1969, Figure 2, p. 15. Reprinted by permission of the publisher.

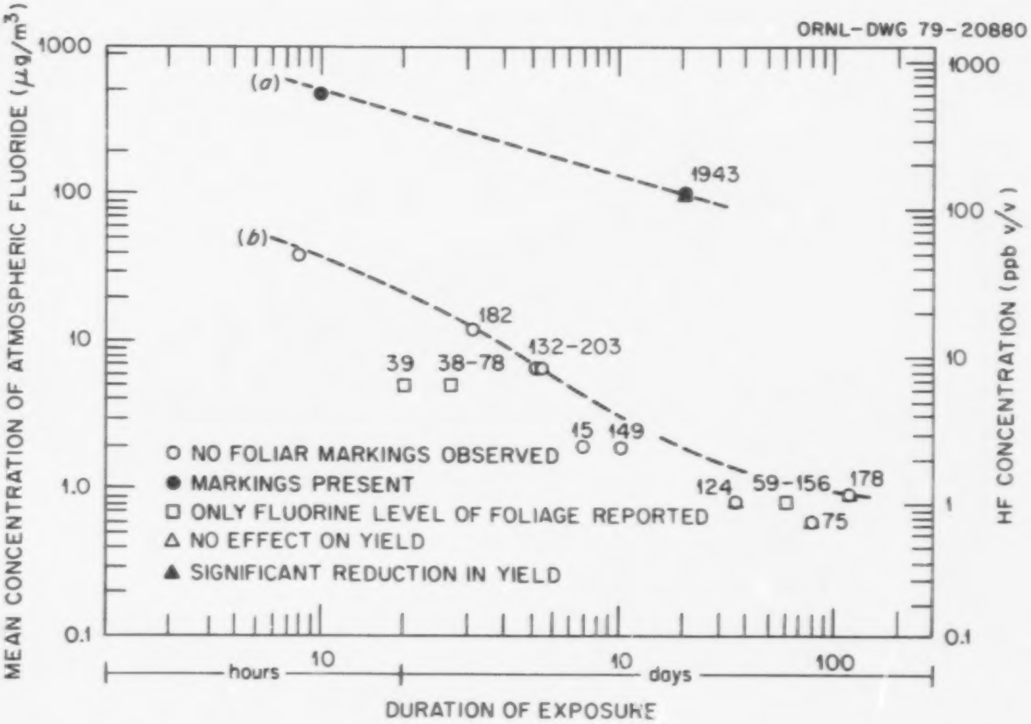

Figure 4.9. Relation of concentration and duration of exposure to effects of atmospheric fluoride on alfalfa. The numbers at indicated points give the fluoride level (on a dry weight basis in parts per million) of unwashed foliage after exposure to atmospheric fluoride. Source: Adapted from McCune, 1969, Figure 3, p. 16. Reprinted by permission of the publisher.

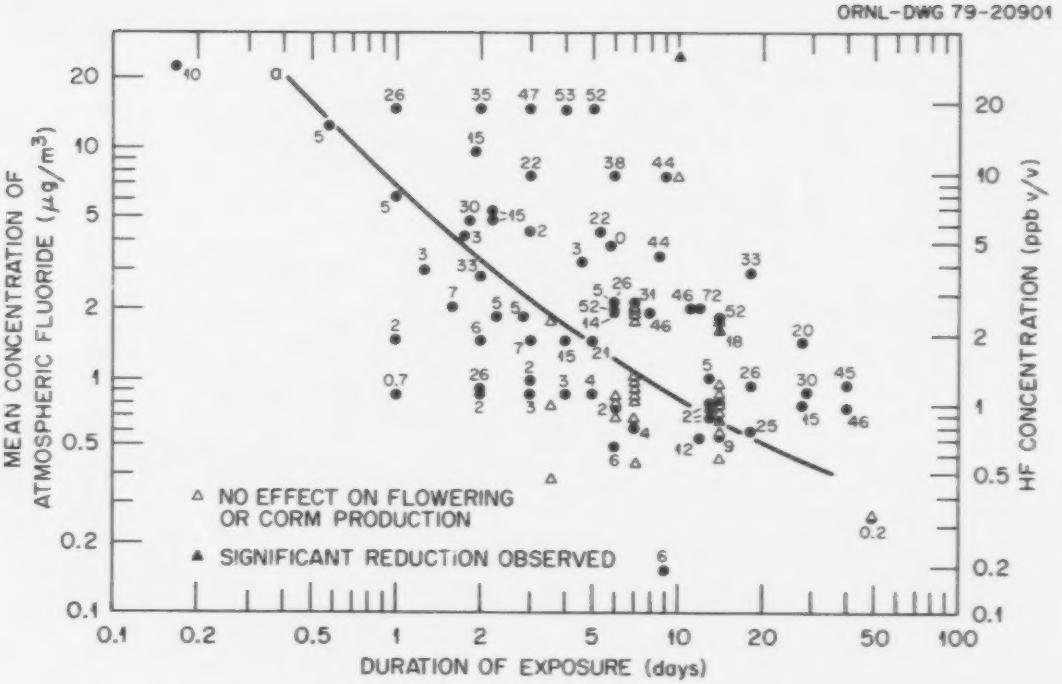

Figure 4.10. Relation of concentration and duration of exposure to effects of atmospheric fluoride on gladiolus. The numbers at indicated points represent percent of leaf area necrosis induced by atmospheric fluoride. Source: Adapted from McCune, 1969, Figure 6, p. 19. Reprinted by permission of the publisher.

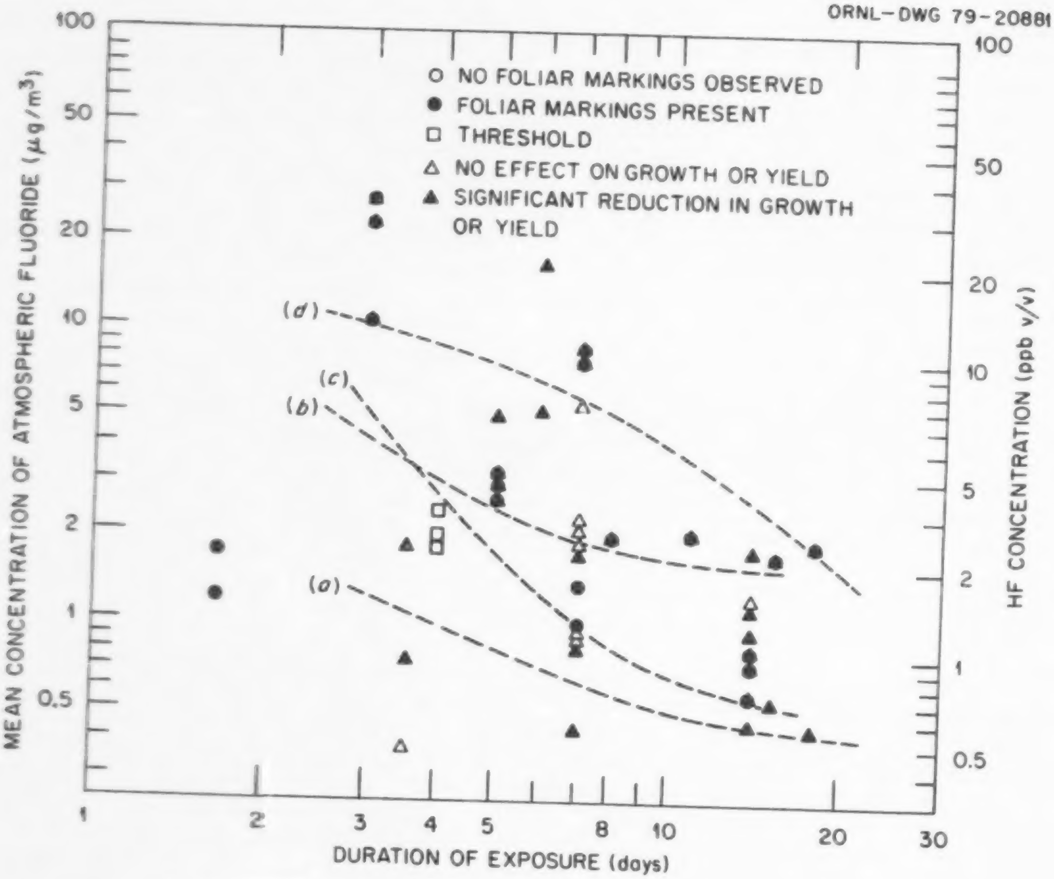

Figure 4.11. Relation of concentration and duration of exposure to effects of atmospheric fluoride on sorghum (Milo maize). Source: Adapted from McCune, 1969, Figure 4, p. 17. Reprinted by permission of the publisher.

fluoride below which no effects are observed (McCune, 1969; National Academy of Sciences, 1971). In Figures 4.8-4.11, the dashed lines represent maximum air concentrations of fluoride that cannot be exceeded during specified time intervals without causing plant injury. For tomato (Figure 4.8), line *a* represents limits above which foliar markings occur, whereas line *b* represents limits above which reduction in quality or quantity of tomato fruits occurs. For alfalfa (Figure 4.9), line *a* represents fluoride concentrations in air above which leaf markings occur; line *b* represents concentrations which, if exceeded, would lead to the accumulation of tissue fluoride greater than 35 ppm. (At the time of publication, 35 ppm was used as an air quality standard in Montana and New York.) For gladiolus (Figure 4.10), a plant very susceptible to fluoride injury, the line represents concentrations in air which, if exceeded, damage more than 5% of the leaf area. If damage greater than 10% occurs, there is a significant reduction in flower and corn yields. The data plotted for sorghum (Figure 4.11) illustrate the difficulty in establishing criteria. Lines *a* and *c* represent levels in air that define vegetative markings and reductions in yield, respectively, in a fluoride-susceptible variety. Lines *b* and *d* are similarly defined for a resistant variety.

One problem with the graphs presented by McCune is that the duration of the exposure periods used for intermittent exposures is the sum of the periods in which treatment took place, not the length of the entire exposure period. It is difficult to use these curves for establishing standards or determining the relationship between ambient concentrations and injury because the relationship in the field has to be based on average concentrations (not just when the pollutant is blowing from the source) over the total duration of the exposure period.

Modern studies of the effects of fluoride on growth and productivity of agricultural plants are usually extrapolations of the results of experimental fumigations to field situations. Large-scale field studies are seldom made because of the difficulties of controlling variables, the uncertainties of applying the results to other sites and crops, and the excessive cost (Weinstein and McCune, 1971). However, field studies are still occasionally performed to evaluate special environmental circumstances, such as the presence of a fluoride-emitting factory. In one such study made near Dresden, Dässler (1971) measured the yield of winter wheat as a function of distance from a hydrofluoric acid factory. He observed reductions in yield of 12.5%, 21.6%, and 25.5% at distances of 2000 m, 1700 m, and 1100 m, respectively, from the factory, compared with the yield at 2700 m. Emissions from the factory varied during the test but frequently exceeded 10 μg of fluoride per cubic meter and occasionally exceeded 50 μg of fluoride per cubic meter. Decreased yields or damaged fruit were also observed at distances up to 6 km for fruit trees (particularly pear, sweet cherry, apple, and plum) and tomatoes.

4.3.3.2 Fluorides and Pollution — Fluoride content is increased in plants growing in the vicinity of fluoride pollution sources, and as a result, growth and productivity of these plants are inhibited. Because of the extensive mining of large phosphate deposits in Florida (over 30 years) and the subsequent release of fluoride to the atmosphere, vast citrus and gladiolus crops, as well as the truck crops in the area, have been damaged (Thomas and Alther, 1966). The expansion of the aluminum industry in the western United States and Canada has, likewise, produced significant fluoride pollution in the surrounding area. Thomas and Alther (1966) cited cases in California of industrial fluoride pollution damage to citrus fruits and wine grapes. Pines and gladiolus in the northwestern United States also have been damaged by fluorides produced from aluminum factories. Fluoride pollution from a phosphate reduction plant in Georgetown Canyon, Idaho, killed about 200 acres of Douglas fir trees (Treshow, Anderson, and Harner, 1967). Needles from trees that eventually died contained an average of 274 ppm fluoride, while needles from trees with no visible symptoms of injury contained 150 ppm fluoride. Needles from control area trees contained only 24 ppm fluoride.

Fluoride injury can occur over an extensive area. Conifers in an area of about 69,000 acres in northwestern Montana showed fluoride damage and contained greater than control levels of fluoride (Carlson, 1973). Emissions from the Anaconda Aluminum Company smelter were determined to be the source of the fluoride.

Gilbert (1973) reviewed the sensitivity of lichens to fluoride pollution and documented several instances where industrial point sources of fluoride pollution resulted in the loss of lichen flora. Fluoride contents of lichens may vary considerably, and the content can be decreased by washing or by rains. Toxicity symptoms include chlorosis and necrosis.

Only a few literature comments are available concerning the recovery of woody plants after field exposure to high fluoride concentrations. After a spill-related release of fluoride in New Jersey in May, peach trees (completely defoliated) and Douglas firs recovered and appeared near normal by August. Norway and blue spruce showed necrosis and abscission and did not show regrowth; the buds did not emerge from their already dormant condition. Needles of Austrian and Scotch pine did not abscise, but new growth did not appear (Rhoads and Brennan, 1975).

4.3.3.3 Growth Effects — Concentrations of fluoride producing high tissue fluoride concentrations and extensive leaf damage will also reduce the growth of the plant (Thomas and Alther, 1966; Treshow, 1971). Numerous examples of this behavior are reported. Linear growth enhancement occurs in some plants at low fluoride concentrations. Fumigation of Koethen sweet orange trees increased linear growth with no increase in leaf number, indicating spindly growth (Matsushima and Brewer, 1972). Stimulation of shoot growth with hydrogen fluoride fumigation (2 ppb) occurs in some rose varieties; however, the stems were weak and therefore the plants were more fragile (Brewer, Sutherland, and Guillemet, 1967). Hitchcock et al. (1971) found that both the wet and dry weight of alfalfa and orchard grass increased with fluoride exposure (six to eight days at the relatively high levels of 16 to 17 μg of fluoride per cubic meter). Benedict, Ross, and Wade (1964), however, found that alfalfa, orchard grass, chard, and romaine lettuce showed no injury symptoms, no significant growth reduction, and no enhancement when continuously fumigated at <1 $\mu g/m^3$ for 80 to 120 days. Treshow and Harner (1968) found significant increases in bean weight with fumigations of 2.3 μg of fluoride per cubic meter for 21 days. The problems of intermittent versus continuous exposure, exposure duration, and species variability are discussed in Sections 4.2.4.1 and 4.3.3.1.

Another somewhat controversial type of injury discussed in the literature is hidden injury. Hidden or invisible injury to a plant caused by a pollutant is defined as interference with growth and normal functioning without the appearance of visible lesions or symptoms (Thomas, 1958, 1961). This concept is of direct concern to agriculturalists interested in optimizing plant yield because data must be obtained to determine if such injury occurs with the levels of fluoride found in the environment. Data supporting the hidden injury concept for fluorides are rare, however.

Hill (1969) summarized long-term studies as follows: in a ten-year-greenhouse study, orchard grass, brome grass, alta fescue, alfalfa, red clover, barley, onions, gladioli, celery, and other species were grown to the normal harvest stage in filtered air, the ambient air near an industrial plant, and hydrogen fluoride at concentrations well above those of the ambient air. No growth reductions were measured except for gladiolus where leaf destruction was severe. In similar studies with raspberries, strawberries, corn, tomatoes, prunes, apricots, and peaches, insufficient fruit was produced for reliable yield measurements, but determinations were made of the overall plant growth, dry weight, terminal growth, girth, number of runners, and other growth indices. The ambient atmosphere had no effect on growth of these crops and hydrogen fluoride caused an effect only when visible injury was produced.

Exposure of gladiolus, a sensitive plant, to low levels of fluoride caused visible injury at the same rate as photosynthesis decreased (Table 4.14) (Thomas, 1958). Fumigation with higher concentrations of fluoride produced a larger decrease in photosynthesis, but recovery occurred after exposure, and the final decrease in photosynthesis was proportional to the damaged leaf area. Fluorides that accumulated in the uninjured portions of the leaf during exposure were translocated to the margins of the leaf during the recovery period. Estimates of the extent of decrease in photosynthesis during high-exposure periods, not accountable by leaf area damage, were only a few percent of the total photosynthesis of the crop. Thus it appears that hidden injury does not occur in gladiolus. Hill (1969) presented similar data for gladioli, strawberries, tomatoes, apricots, and corn. He concluded that the treatment either did not have any significant effect on apparent photosynthesis or the effect could be accounted for by an equal amount of leaf necrosis. With corn, chlorotic mottling developed rather than necrosis, and the measured reduction in photosynthesis was attributed to reduced photosynthesis in the chlorotic tissue. Hill et al. (1958) exposed tomatoes, a crop relatively resistant to fluoride, to rather high airborne fluoride concentrations (1.8 to 6.5 $\mu g/m^3$ for the Moscow variety and 3.4 to 73 $\mu g/m^3$ for the Loran Blood variety). This exposure caused high tissue levels of fluoride. No leaf symptoms were observed in Moscow tomatoes and only a trace to 2% leaf area damage in the Loran Blood variety. No difference in respiration or in average carbon dioxide assimilated per hour per plot was detected between treatment and control plots. Consequently, the authors concluded that no hidden injury occurred even with the high fluoride concentrations used.

TABLE 4.14. EFFECT OF FUMIGATION WITH RELATIVELY LOW CONCENTRATIONS OF HYDROGEN FLUORIDE ON THE PHOTOSYNTHESIS OF PLANTS

Plant	HF fumigation			Photosynthesis reduction[b] (%)	Leaf area destroyed (%)
	Duration[a] (hr)	Concentration (ppb)			
		Maximum	Average		
Fruit trees	183	9.0	2.6	14	10
	39	15.0	4.8	2	<5
	8	4.0	3.9	0	0
Gladiolus					
Surfside	53	11.3	6.5	15	15
	44	9.8	6.4	28	30
	205	13.2	4.5	45	44
	128	33.4	5.7	20	22
Alladin	30	5.1	3.9	0	3
Algonquin	111	7.1	4.3	2	3
Commander Koehl	42	10.2	5.5	0	3
Mixed grain	10	200	145	0	Trace
Cotton	77	692	237	0	Trace
	138	33	17	0	0

[a] Fumigations 4 to 9 hr/day.
[b] Fumigated plot per check plot.

Source: Adapted from Thomas, 1958, AGRONOMY JOURNAL, Volume 50, page 547, by permission of the American Society of Agronomy.

Reduction in top growth of fluoride-sprayed citrus trees was correlated with reduction in leaf size and leaf drop (Brewer, Sutherland, and Guillemet, 1969). This suggests that hidden injury may not occur in citrus either.

However, other evidence suggests that hidden injury occurs in some species. Treshow, Anderson, and Harner (1967) reported decreased growth, but no fluoride necrosis, in Douglas fir trees growing near a phosphate reduction plant. Where necrosis did occur, it was associated with still greater growth suppression. Two years after fluoride emissions had stopped, tissue fluoride concentrations averaged 150 ppm in the area of decreased growth. The data indicate that fluoride levels were more than twice as high when the plant was in operation. At low atmospheric fluoride concentrations, enhancement of shoot growth occurred. Dendrochronological data show that air pollution (presumably fluorides) from a fertilizer plant decreases radial growth in Scots pine (Havas and Huttunen, 1972). It is not clear that this occurred in the absence of visible injury, however, but the reduction in growth was detected prior to death or serious damage. Younger trees are affected more than older trees. In other studies, continuous hydrogen fluoride fumigations at 0.55 and 2.0 µg of fluoride per cubic meter produced deformed strawberry fruits and decreased their average fresh weights. No visible injury occurred on the foliage, but visible effects were observed on the fruit (Pack, 1972).

4.3.3.4 Interactions with Other Pollutants and Pathogens — There are a variety of other important but, at present, unanswered questions concerning the effect of fluorides on plants. Interactions with other biological and physical components of the ecosystem are especially important.

Few data were found on the effects on plants exposed to combinations of fluorides and other pollutants. Effects of hydrogen fluoride and sulfur dioxide were additive with respect to reduction of linear growth and leaf area of Koethen orange and Satsuma mandarin (Matsushima and Brewer, 1972). With the concentration of hydrogen fluoride and sulfur dioxide used, necrosis did not occur on Koethen orange. Effects of sulfur dioxide and hydrogen fluoride on chlorosis in Satsuma mandarin were less than additive. Bennett and Hill (1974) observed that the inhibition of photosynthesis in alfalfa by nitrogen dioxide plus hydrogen fluoride was additive; inhibition with sulfur dioxide plus hydrogen fluoride was slightly greater than additive. Both barley and sweet corn showed increased foliar damage when fumigated with hydrogen fluoride (approximately 0.00075 ppm) plus low concentrations of sulfur dioxide (0.08 ppm) as compared with either sulfur dioxide or hydrogen fluoride alone (Mandl, Weinstein, and Keveny, 1975). Beans were not injured at any of the concentrations used. Fluoride accumulation by leaves of barley and corn was less for hydrogen fluoride plus sulfur dioxide than for hydrogen fluoride alone, the result possibly relating to the effectiveness of sulfur dioxide in producing stomatal closure. Zimmerman and Hitchcock (1956) observed that some species most susceptible to sulfur dioxide (chicory, eggplant, geranium, pigweed, tobacco, dandelion, and celery) are resistant to hydrogen fluoride.

There are few data on the relationship between susceptibility of a species to parasitic invasion and fluoride injury of the species. Wentzel (as cited in ten Houten, 1972) observed that fluoride-injured spruce contained many more galls of the spruce-gall aphid than uninjured spruce. Although an injured plant is often thought to be more susceptible to injury from another agent, this is not always the case. Examples with other pollutants illustrate that increased pollutant levels may decrease parasitic injury and that parasitic attack may decrease pollutant injury. Few reports detail the interactions between fluorides and parasites. Exposure of bean plants to 7 to 10 μg of hydrogen fluoride per cubic meter reduced the extent of infection with powdery mildew (McCune et al., 1973, as cited in Treshow, 1975). Fluoride reduced the number of rust uredia found on bean and reduced early blight of tomato caused by *Alternaria solani* when hydrogen fluoride fumigation occurred prior to inoculation with the fungus. Fluorides did not affect the severity of halo blight caused by *Pseudomonas phaseolicolus* or of late blight caused by *Phytophthora infestens*.

Exposure of young pinto beans to fluorides increased viral activity as tissue fluoride concentrations of cut leaves and intact plants increased to about 300 ppm and 500 ppm respectively (Treshow, 1975). Above these concentrations, virus activity decreased below control values. The extreme variation observed in such lesion assays and the influence of environmental factors make it unlikely that variations in virus infectivity due to field exposure of plants to fluorides could be detected.

4.3.4 Cytogenetic Effects

There is evidence that fluorides can be mutagenic. Fumigation of tomato plants with hydrogen fluoride at 3 $\mu g/m^3$, a concentration that did not produce visible injury until 11 days of exposure, induced chromosomal aberrations during mitosis and meiosis (Mohamed, Smith, and Applegate, 1966). The aberrations observed were chromosomal bridges and fragments. Solutions of 10 m*M* sodium fluoride caused chromosomal breakage and stickiness in mitotic cells of onion root tips (Mohamed, Applegate, and Smith, 1966). Fumigation of microsporocytes from maize plants with 3 $\mu g/m^3$ hydrogen fluoride produced no visible injury, but the treated microsporocytes contained a higher frequency of asynaptic regions and total aberrations than microsporocytes from control plants (Mohamed, 1970). An increase in aberrations occurred with increased exposure to hydrogen fluoride (four- to ten-day exposures). Contrary to experiments with onion and tomato, hydrogen fluoride partially inhibited cytokinesis in maize.

Root tip cells of germinating barley treated with sodium fluoride and hydrogen fluoride at 10 m*M*, 0.1 m*M*, and 1 μ*M* showed several anomalies — chromosomal bridges with and without fragments, polyploid cells, binucleate cells, ball metaphases, micronuclei, tripolar and multipolar anaphases, and fusion of telophasic groups of chromosomes (Bale and Hart, 1973*a*). Treatment of barley coleoptiles with 1%, 4%, or 6% sodium fluoride alone or with dimethyl sulfoxide (DMSO) did not produce injury symptoms or inhibit subsequent growth of the barley seedlings (Bale and Hart, 1973*b*). However, the incidence of chromosomal aberrations in the pollen mother cells and percentage of pollen aborted from treated plants increased with fluoride concentration and was significantly greater than that from untreated plants. At low fluoride concentrations, DMSO increased the number of abnormalities. No chlorophyll mutations were observed in the progeny, suggesting that fluoride does not induce specific mutations. No chlorophyll mutations were observed in the M_2 generation of barley obtained from seed that had been soaked in 10 m*M* sodium fluoride (Jagannath and Bhatia, 1974). Fluoride did, however, significantly increase the mutagenic effects of gamma radiation under aerobic conditions.

Increased exposure time (1 to 24 hr) and concentration of fluoride (1 to 1000 ppm) produced increased mitotic and meiotic aberrations in *Vicia faba* (Hakeem and Shehab, 1970). The abnormalities were similar to those observed by Bale and Hart (1973*a*) in barley.

The mechanisms by which fluoride causes chromosomal alterations in plants have not been unequivocally established (Chang, 1975); probably, processes similar to those discussed for microorganisms are involved (Section 3.2.1). Chromatid breakage and stickiness have also been suggested (Mohamed, 1970). The production of abnormalities when plants are treated with fluoride concentrations that do not inhibit growth or produce visible injury means that fluoride can be considered a mutagenic agent.

SECTION 4

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SECTION 5

BIOLOGICAL ASPECTS IN DOMESTIC AND WILD ANIMALS

5.1 SUMMARY

Animals are exposed to fluorides in various forms and concentrations by ingestion of food and water, receiving only minimal amounts from the atmosphere. Ingestion is the principal form of uptake by most animals. Normally, inhalation and skin absorption contribute negligible amounts to the total fluoride intake. In animals, fluorides accumulate in mineralized tissues such as bones and teeth.

Mammals usually excrete fluorides rapidly from the body, with urinary excretion accounting for the major portion of elimination. Small amounts are excreted in feces, saliva, and perspiration. Both rapid elimination and deposition of fluorides in mineralizing tissues provide methods of reducing fluoride toxicosis.

Fluoride-induced effects in animals generally result from the ingestion of excessive amounts. Some specific enzymatic activity is inhibited by excessive fluoride body levels. Impaired ability to metabolize fat (suggesting fatty acid oxidase inhibition) may occur in animals with fluoride toxicosis, and carbohydrate metabolism may also be altered. During periods of excessive fluoride intake, bone alkaline phosphatase activity increases.

Both inorganic and organic fluorides produce toxic effects in some insects. General effects include lethal and sublethal toxicosis, decreased reproduction, and changes in mutation frequencies.

Some aquatic organisms are sensitive to fluorides. This sensitivity is related to environmental acclimatization and species. For example, crustaceans are tolerant of high fluoride levels in seawater, while the most sensitive aquatic organisms appear to be filter-feeding molluscs. Environmental concentrations of less than 1.5 ppm do not seem to have harmful effects on aquatic species.

Ingestion of excessive fluoride by birds induces a reduced growth rate and inhibits specific enzymes such as neurotoxic esterase; however, it enhances the activity of other enzymes such as plasma alkaline phosphatase. Both acute and chronic toxicity occur as a result of exposure to excessive fluoride levels. Dental and bone lesions are the primary clinical signs of chronic fluoride toxicosis. Loss of appetite and hemorrhages of the gastrointestinal tract are associated with acute fluoride toxicosis.

In mammals, developing teeth are sensitive to fluoride. During the formative stage, dental fluorosis is a major symptom of excessive fluoride exposure. Bone lesions, lameness, and reduced feed intake are induced by prolonged periods of high fluoride ingestion. Dairy cattle are the domestic animals most sensitive to fluoride toxicosis. Therefore, fluoride

levels that are safe for dairy cattle are considered safe for other species of domestic livestock.

5.2 INSECTS

5.2.1 Metabolism

Data concerning the metabolism of fluoride-containing compounds by insects are limited; most are found primarily in fluoride toxicology studies.

5.2.1.1 Uptake — Insects usually take up fluorides by ingestion. By feeding on substances on which fluoride-containing compounds are deposited or by cleaning itself of adhering fluoride compounds, an insect will ingest varying quantities of these compounds. Some fluoride-containing insecticides, such as sodium fluoride, are thought to be directly absorbed by some insects through the membranous areas of the cuticle, at the junction of the head and thorax, and the coxa (Griffiths and Tauber, as cited in Metcalf, 1966).

In tests designed to study fluoride uptake, eggs of *Schistocerca gregaria* and *Tenebrio molitor* were exposed at 30°C to 11 ppm ^{35}S-labeled sulfuryl fluoride for 4, 8, 16, 24, 32, or 62 hr (Outram, 1967). Penetration was mainly through the micropylar complex in *S. gregaria* eggs and through the general surface of the chorion in *T. molitor* eggs. Both the total uptake and irrecoverable fraction of the fumigant varied with the stage of embryonic development (Figure 5.1). The rapid uptake pattern for *T. molitor* eggs indicated that uptake was governed by the rate of fixation of the fumigant in tissues, whereas the slower uptake of *S. gregaria* eggs may have represented a toxicosis process of the embryonic tissues.

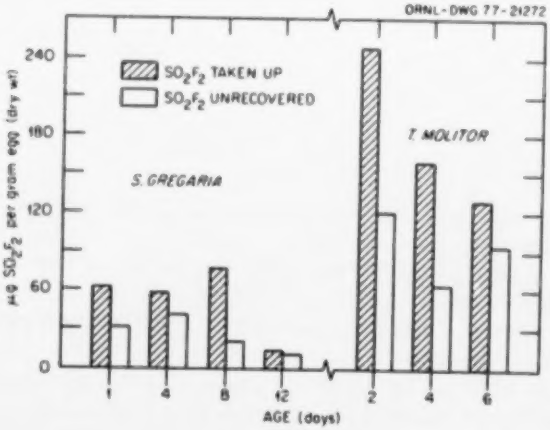

Figure 5.1. The amount of $^{35}SO_2F_2$ present in the eggs of *Schistocerca gregaria* and *Tenebrio molitor* at different stages of embryonic development. Source: Adapted from Outram, 1967, Figure 2, p. 258. Reprinted by permission of the publisher.

5.2.1.2 Accumulation — Dewey (1973) found fluoride accumulation in insects collected near an aluminum reduction operation. Fluoride levels in insects collected near the operation ranged from 2 to 20 times (pollinators) as high as fluoride levels in control insects collected 50 miles from the aluminum operation. Four groups of insects were collected: pollinators, predators, foliage feeders, and cambial-region feeders. Table 5.1 lists the fluoride levels found in control insects, and Table 5.2 lists the fluoride levels in test insects. High fluoride levels in the predatory insects indicated that fluorides could be accumulated not only from foliage feeding and by respiratory processes but through the food chain.

No data were found concerning the distribution, half-life, or elimination of fluoride in insects.

TABLE 5.1. FLUORIDE LEVELS IN CONTROL INSECTS

Insect	Fluoride level (ppm dry wt)[a]
Larch casebearer (*Coleophora laricella*)	16.5
Engraver beetle (*Ips* sp.)	11.5
Honeybee (*Apis mellifera* L.)	10.5
Damselfly (*Argia* sp.)	9.2
Grasshopper (*Melanoplus* sp.)	7.5
Bumblebee (*Bombus* sp.)	7.5
Red turpentine beetle (*Dendroctonus valens* LeConte)	4.8
Flatheaded borers (mixed Buprestidae)	3.5

[a] Extract of whole insect.

Source: Adapted from Dewey, 1973, Table 1, p. 180. Reprinted by permission of the publisher.

5.2.2 Effects

5.2.2.1 Toxicity — Fluoride compounds, such as sodium fluoride and iron fluorides, have been used for insect control for the last 100 years (Metcalf, 1966). Inorganic fluorides replaced arsenical insecticides for agricultural uses; however, the use of inorganic fluorides has diminished in recent years. Many organic fluoride-containing compounds, such as fluoroacetic acid, fluoroethyl acetate, and methylsulfonyl fluoride, have also proven to be effective insecticides (National Academy of Sciences, 1971). The effects of fluoride compounds on insects are general lethal and sublethal toxicity, decreased reproduction, and changes in mutation frequencies.

TABLE 5.2. FLUORIDE LEVELS IN TEST INSECTS

Insect	Fluoride level (ppm dry wt)[a]
Pollinators	
Bumblebee (*Bombus* sp.)	194.0 406.0
Honeybee (*Apis mellifera*)	221.0
Mixed Hymenoptera	585.0
Sphinx moth (*Hemaris* sp.)	394.0
Wood nymph butterfly (*Cercyonis* sp.)	58.0 144.0
Skipper butterfly (*Erynnis* sp.)	146.0 81.3
Mixed Syrphidae	140.0
Predators	
Ants (mixed Formicidae)	170.0
Robberfly (mixed Asilidae)	82.9
Ostomids (*Temnochila* sp.)	53.4
Dragonfly (mixed Anisoptera)	24.8
Damselfly (*Argia* sp.)	21.7
Long-legged fly (*Medeterus* sp., larvae)	10.2
Ostomidae (larvae)	6.1
Foliage feeders	
Arctiidae (larvae)	255.0
Notodontidae (larvae)	168.0
Weevil (mixed Curculionidae)	48.6
Grasshopper (*Melanoplus* sp.)	31.0 29.0
Larch casebearer (*Coleophora laricella*)	25.5
Cicada (Cicadidae)	21.3
Cambial region feeders	
Engraver beetle (*Ips* sp.)	52.5
Flatheaded borer (mixed Buprestidae)	20.0
Red turpentine beetle (*Dendroctonus valens*)	11.5
Douglas fir beetle (*Dendroctonus pseudotsugae* Hopkins)	9.4
Flatheaded borer (mixed Buprestidae, larvae)	8.5
Miscellaneous insects	
Long-horned beetle (mixed Cerambycidae)	47.5
Click beetle (mixed Elateridae)	36.0

[a] Extract of whole insect.

Source: Adapted from Dewey, 1973, Table 2, p. 180. Reprinted by permission of the publisher.

5.2.2.1.1 General toxicity — Sodium fluoride administered by ingestion was toxic to *Mamestra brassicae* caterpillars, *Scotia segetum* caterpillars, larvae of *Leptinotarsa decemlineata*, *Lymantria dispar* caterpillars, and *Hyphantria cunea* caterpillars (Weismann and Svataráková, 1974). The LD_{50} for each species, based on the milligrams of sodium fluoride administered and the kilograms of living weight of the insect, is given in Table 5.3. The most resistant insect was *L. decemlineata*. The differences in susceptibility were attributed to the bonding of fluoride ions to calcium to form insoluble calcium salts. In this manner, fluoride may have restricted the function of calcium in the insect and induced death. The LT_{50} for each insect is given in Table 5.4.

TABLE 5.3. SODIUM FLUORIDE TOXICITY TO SELECTED INSECT SPECIES

Insect species	Instar	Average weight (g)	LD_{50} (mg NaF)	LD_{50}/kg living weight (mg NaF)
Scotia segetum	V	0.416	0.078	187.5
	IV	0.269	0.027	102.7
Mamestra brassicae	V	0.502	0.052	103.6
	III	0.118	0.031	262.7
Leptinotarsa decemlineata	IV	0.198	0.050	252.5

Source: Adapted from Weismann and Svataráková 1974, Table 1, p. 848.

TABLE 5.4. LT_{50} VALUES COMPUTED FOR VARIOUS DOSES OF SODIUM FLUORIDE AND DEVELOPMENT STAGES OF SELECTED INSECT SPECIES

NaF dose consumed (mg)	LT_{50} value (days)						
	Scotia segetum, instar V	*Mamestra brassicae*		*Leptinotarsa decemlineata*		*Hyphantria cunea*, instar V	*Lymantria dispar*, instar V
		Instar IV	Instar V	Instar III	Instar IV		
0.25	1.8		1.5	1.75	2.55		
0.1	2.5	1.8	2.45	2.45	2.7	1.7	1.8
0.05	5.5	2.6	3.2	2.15	3.15	4.6	5.6
0.025	12.3	3.4	11.0	3.1	4.6	9.2	10.3

Source: Adapted from Weismann and Svataráková, 1974, Table 2, p. 849.

The uptake of 0.1 mg of sodium fluoride by *S. segetum* caused a decrease in food uptake, movement reduction, shrinking, body blackening, and finally death (Weismann and Svataráková, 1973). The average life span of the caterpillar decreased in proportion to the sodium fluoride level administered. Sodium fluoride was shown to be an effective stomach poison as well as contact poison for the American roach (*Periplaneta americana*) (Sweetman, 1941).

Sulfuryl fluoride was an effective insecticide for drywood termites (*Cryptotermes brevis*); temperature influenced the toxicity level causing a higher mortality rate at 80°F than at 70°F (Table 5.5) (Bess, 1971). Minnick, Kerr, and Wilkinson (1972) also reported that sulfuryl fluoride was an effective control for *C. brevis*.

TABLE 5.5. MORTALITY OF *CRYPTOTERMES BREVIS* EXPOSED TO SULFURYL FLUORIDE FOR 3 hr IN TEMPERATURE-CONTROLLED FUMIGATION CHAMBERS

Dosage (oz/1000 ft^3)	Exposed in microscope slide cages			Exposed in sealed block cages		
	Number of termites in tests	Number of termites dead after 5 days	Mortality (%)	Number of termites in tests	Number of termites dead after 5 days	Mortality (%)
		Exposure data at 80°F				
7.5	247	7	3	20	1	5
9.0	348	79	23	49	11	22
10.5	248	191	77	80	16	20
11.5	100	89	89	30	6	20
12.0	247	238	96	80	37	46
14.0	98	97	99	30	18	60
		Exposure data at 70°F				
8.0-10.0	198	4	2			
11.5-12.0	198	37	19			
13.0-13.5	199	102	51			
15.5-16.0	299	178	60			
17.5-19.0	200	197	98			
0 (controls)	348	5	1	206	5	2

Source: Adapted from Bess, 1971, Table 1, p. 12. Reprinted by permission of the publisher.

The toxicity of various fluorine compounds to *Bombyx mori* and *Apis mellifera* is presented in Table 5.6. The insecticidal properties of these compounds apparently depend on their fluorine content (Metcalf, 1966). These and other inorganic fluorine compounds act principally as stomach poisons, owing their activity to inhibition of magnesium-containing enzymes by fluoride ions.

Many organofluorine compounds are effective insecticides. According to Metcalf (1966), these organic compounds act in one of three ways: (1) as a blocking atom that mimics hydrogen but does not take part in enzymatic reaction, (2) as a reactive atom that permits the compound to react with a vital enzyme, and (3) as a halogen providing a stable lipophilic group with an aliphatic or aromatic nucleus. Various organofluorine compounds containing phosphorus (Joshi and Tholia, 1973) and α,α,α-trifluoroacetophenone oxime carbamates (Rosenfeld and Kilsheimer, 1974) have insecticidal activity. Under experimental conditions, fluoroacetamide was more toxic against *Aphis fabae*, *Brevicoryne brassicae*, *Myzus persicae*, and the eggs and larvae of *Pieris brassicae* than sodium fluoroacetate (David and

TABLE 5.6. TOXICITY OF INORGANIC FLUORINE COMPOUNDS TO *BOMBYX MORI* AND *APIS MELLIFERA*

Compound	Oral LD_{50} (μg/g)	
	Bombyx mori, 4th instar	*Apis mellifera*
NaF	110-150	60-70
CaF_2		>540
MgF_2	>570	
MnF_2	200-400	
PbF_2	250-400	
Na_2SiF_6	100-130	240
K_2SiF_6	70-100	50
$BaSiF_6$	90-120	60-70
Na_3AlF_6	50-70	60-70
K_3AlF_6	80-100	
$(NH_4)_3AlF_6$	110-140	

Source: Adapted from Metcalf, 1966, Table 1, p. 356. Reprinted by permission of the publisher.

Gardiner, 1958). A 0.001% solution of fluoroacetamide produced a 100% kill of *A. fabae* and *B. brassicae*. As a systemic insecticide, fluoroacetamide was as effective as or slightly superior to sodium fluoroacetate; the LD_{100} in milligrams per kilogram of fresh plant weight for *A. fabae* was 0.09 to 0.9 for fluoroacetamide and 0.7 for sodium fluoroacetate.

5.2.2.1.2 Reproduction — When insects are exposed to poisons there may be, depending on the concentration of the poison and the length of exposure, an inhibition or a stimulation in egg production. A seven-day exposure of *Tribolium confusum* to flour containing 0.01% sodium fluoride produced stimulation of egg production (Johansson and Johansson, 1972). However, egg production was adversely affected by prolonged exposure to flour containing 0.1% sodium fluoride (Table 5.7). Total egg production per female exposed to flour containing 1% sodium fluoride for two and four days was significantly greater than that of the nonexposed controls. However, egg production was decreased in female insects exposed for six days.

Gerdes, Smith, and Applegate (1971) exposed two strains of *Drosophila melanogaster* to 1.3 and 2.9 ppm hydrogen fluoride. Fecundity, hatchability, and male fertility were evaluated in adults raised from these eggs. All three of these parameters were reduced in the flies (Table 5.8), but there was no reduction in female fertility. Reduction of these parameters in *D. melanogaster* exposed to sublethal levels of hydrogen fluoride may be a result of genetic damage caused by the fluoride.

5.2.2.1.3 Mutagenicity — Various fluoride compounds are mutagenic to *Drosophila*, causing recessive sex-linked mutations. Garret and Fuerst

TABLE 5.7. EGG PRODUCTION BY *TRIBOLIUM CONFUSUM* PER FIVE-DAY INTERVAL AFTER EXPOSURE TO SODIUM FLUORIDE LEVELS VARYING FROM 0 TO 0.1%

NaF level (%)		Mean number of eggs per female				Mean total[a]
Days 1-7	Days 7-27	Days 7-12	Days 13-17	Days 18-22	Days 23-27	
0	0	22.2	25.2	24.0	22.0	93.4 ab
Treatment A						
0.0001	0.0001	23.7	24.3	27.7	25.8	101.5 bc
0.001	0.001	18.9	23.8	25.4	26.3	94.4 abc
0.01	0.01	19.6	24.4	26.7	30.6	101.3 bc
0.1	0.1	13.9	20.9	24.7	25.3	84.8 a
Treatment B						
0	0.0001	22.2	22.3	21.1	22.3	87.9 a
0	0.001	20.2	23.0	24.7	25.2	93.1 ab
0	0.01	22.3	25.6	26.4	26.1	100.4 bc
0	0.1	9.1	12.4	15.4	17.7	54.6
Treatment C						
0.0001	0	27.7	29.1	24.5	25.9	107.2 c
0.001	0	26.0	29.5	25.5	26.7	107.7 c
0.01	0	27.0	28.0	31.5	30.4	116.9
0.1	0	10.7	28.0	28.5	28.3	95.5 abc

[a] Means followed by one or more of the same letters are not significantly different from one another.

Source: Adapted from Johansson and Johansson, 1972, Table 1, p. 357. Reprinted by permission of the publisher.

TABLE 5.8. MEAN VALUES OF BIOLOGICAL PARAMETER RESPONSE IN *DROSOPHILA MELANOGASTER* TO ATMOSPHERIC CONTAMINATION BY HYDROGEN FLUORIDE

Treatment duration (weeks)	HF level (ppm)	Fecundity[a]		Hatchability[b]		Male fertility[c]	
		Oregon-r	Yellow white	Oregon-r	Yellow white	Oregon-r	Yellow white
0	0	280	262	82	79.5	100	100
	1.3	280	271	79	79	100	99
	2.9	293	268	78	75.5	99	99
3	0	286	261	79.5	76	100	99
	1.3	242	241	71	70.5	98.5	97
	2.9	222	225	73	71	97	93
6	0	289	270	79	78	100	100
	1.3	196	212	74	71	99	96
	2.9	192	198	65.5	61	92	91

[a] Mean number of eggs from seven egg collections (days 2, 3, 6, 7, 10, 11, and 14).

[b] Mean percentage from four egg collections (days 2, 6, 10, and 14).

[c] Mean percentage.

Source: Adapted from Gerdes, Smith, and Applegate, 1971, Table 1, p. 118. Reprinted by permission of the publisher.

(1974) reported a significant increase in sex-linked recessive lethal mutations in *D. melanogaster* when exposed to perfluorobutene-2; fluoroform; 1,1-difluoroethane; and sulfurchloridopentafluoride. Perfluorobutene-2 produced a recessive mutation rate of 1.78%, compared to a rate of 0.25% for the control. The mutagenic effects of perfluorobutene-2 in combination with oxygen, carbon dioxide, nitrogen, and compressed air were also tested. These results are given in Table 5.9.

TABLE 5.9. RECESSIVE MUTATION RATE IN *DROSOPHILA MELANOGASTER* EXPOSED TO PERFLUOROBUTENE-2 AND PERFLUOROBUTENE-2 IN COMBINATION WITH OXYGEN, CARBON DIOXIDE, NITROGEN, AND COMPRESSED AIR

Gas introduced[a]	Gas atmosphere	Recessive mutation rate (%)
None (atmospheric air control)	100% air	0.21 ± 0
Compressed air	100% air	0.25 ± 0.05
Perfluorobutene-2	10% perfluorobutene-2, 90% air	1.78 ± 0.02
50% perfluorobutene-2	5% perfluorobutene-2, 95% air	1.01 ± 0.04
Oxygen	10% oxygen, 90% air	0.72 ± 0.04
50% perfluorobutene-2, 50% oxygen	5% perfluorobutene-2, 5% oxygen, 90% air	1.23 ± 0.05
Nitrogen	10% nitrogen, 90% air	0.31 ± 0.04
50% perfluorobutene-2, 50% nitrogen	5% perfluorobutene-2, 5% nitrogen, 90% air	1.03 ± 0.04
Carbon dioxide	10% carbon dioxide, 90% air	0.29 ± 0.05
50% perfluorobutene-2, 50% carbon dioxide	5% perfluorobutene-2, 5% carbon dioxide, 90% air	0.91 ± 0.02

[a] The gases were introduced into 100-ml Turner bulbs containing the flies in air at a flow rate of 13 ml/min, which was monitored by Matheson flowmeters. The *Drosophila* males were gassed for 1 min and then kept in the gas atmosphere for 6 min.

Source: Adapted from Garrett and Fuerst, 1974, Table 2, p. 291. Reprinted by permission of the publisher.

Repair of radiation damage in *Drosophila* is influenced by sodium fluoride. Valentin (1971) injected *Drosophila* with 5-m*M* and 10-m*M* solutions of sodium fluoride. A complex response pattern resulted in which both increases and decreases of recombination occurred. Ivashchenko, Grozdova, and Kholikova (1971) also found that sodium fluoride changed the frequency of lethal and sublethal mutations in *Drosophila* when they were irradiated. In the cases where irradiation occurred in air, the change in mutation frequency took place at the stage of mature germ cells. Injection of 0.15 mg of sodium fluoride per fly lowered the frequency of lethal mutations and increased the frequency of sublethal mutations.

5.3 AQUATIC ORGANISMS

5.3.1 Metabolism

5.3.1.1 Uptake — Aquatic organisms can obtain fluoride by ingestion of high-fluoride-containing foods (Wright and Davison, 1975). However, fluoride ions can also enter fish through fluoride-permeable gills (Bielawski, 1971). Hemens and Warwick (1972) stated that the mullet *Mugil cephalus*, the crab *Tylodiplax blephariskios*, and the shrimp *Palaeman pacificus* take up fluoride mainly from water, not from foods.

5.3.1.2 Accumulation and Distribution — Mullet, crab, and shrimp exposed for 72 days to 52 ppm fluoride, added as sodium fluoride to the water, had a higher fluoride tissue concentration than the control organisms exposed to 1.05 ppm fluoride (Hemens and Warwick, 1972). Mullet accumulated the highest concentration at 7743 ppm fluoride; the control mullet had 141.8 ppm fluoride. Shrimp accumulated 3116 ppm fluoride, and the mud crab accumulated 1414 ppm fluoride. The control groups accumulated 106 ppm and 169.6 ppm respectively.

Fluoride concentration in muscle tissues of carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) varied with the fluoride concentration (0.1 to 25 ppm) in the water in which the fish were placed (Neuhold and Sigler, 1960). High fluoride levels in water produced fluoride tissue levels of 20.83 ppm, whereas low fluoride water levels gave fluoride tissue concentrations of 2.95 ppm. Fluoride levels in the osseous tissues were related to the fluoride water concentrations and the length of exposure. Fluoride accumulation by bone was assumed to result from an enzymatic process. Fluorides tend to occur in fish blood in both an unassociated ionic form and associated with another ion or molecule. Because of its high electronegativity the fluoride ion is more likely to be associated with the electropositive elements.

Blue crabs (*Callinectes sapidus*) from relatively unpolluted water (0.5 to 1.5 ppm fluoride) contained fluoride levels that varied for the different kinds of tissues sampled (Table 5.10) (Moore, 1971). Gills were the only tissue that showed a significant difference in the amount of fluoride accumulated between large and small crabs; a possible accumulation mechanism associated with the gills may be responsible for this difference. The exoskeleton, gills, hepatopancreas, and muscle tissues all accumulated significant amounts of fluoride in crabs exposed to water containing 20 and 100 ppm fluoride for 90 days, 200 ppm for 22 days, and 400 ppm for 70 days. Crab muscle tissue accumulated 50 ppm fluoride (dry weight) when exposed to water containing 20 ppm fluoride. This behavior indicates that crab muscle can accumulate sufficient fluoride from polluted water to be a potential health problem for humans if ingested at a higher rate. Over a 20-day period in fluoride-free water following a 30-day exposure time, tissues readily released accumulated fluoride and most returned to near normal tissue levels (Figure 5.2).

TABLE 5.10. FLUORIDE CONCENTRATIONS IN DRIED TISSUES OF CRABS FROM NATURAL, UNPOLLUTED WATERS

Tissue	Fluoride concentration (ppm)	
	Small crabs (53-81 mm)	Large crabs (100-117 mm)
Exoskeleton	267	300
	272	325
	319	300
	295	310
	290	256
Average	288.6	298.2
Gills	158	210
	180	225
	145	280
	174	190
	165	360
Average	164.4	253.0
Hepatopancreas	20	28
	15	20
	21	20
	18	24
	23	18
Average	19.4	22.0
Muscle	13	10
	8	16
	10	7
	10	9
	12	8
Average	10.6	10.0

Source: Adapted from Moore, 1971, Table 1, p. 5. Reprinted by permission of the publisher.

In other studies, fluoride accumulated in the skeleton and skin of fish and the exoskeleton of aquatic invertebrates (Wright and Davison, 1974). Tissue and seawater samples were taken from the inflow filter at a power station in England and from offshore points several kilometers away from the plant. In fish bone, fluoride deposition was thought to occur by fluoride-anion exchange in the hydroxyapatite complex. In exoskeletons, the process of fluoride accumulation was by calcium fluoride precipitation. A comparison of fluoride levels in fish taken from the inflow filter and off shore indicated that there were no differences in fluoride concentrations; however, variability of the data was too large to make the comparison significant (Wright and Davison, 1975). Tissue fluoride concentrations for the species sampled are presented in Table 5.11. Fluoride accumulated rapidly in tissues of the mollusc *Mytilus edulis*, probably because of the large turnover of seawater and suspended particles containing fluoride. Shore crabs (*Carcinus maenas*) exposed to various fluoride concentrations accumulated fluoride in proportion to the fluoride level of the seawater (Figure 5.3).

5.3.1.3 Excretion — Very little information is available concerning excretion of fluoride by aquatic organisms. Neuhold and Sigler (1960)

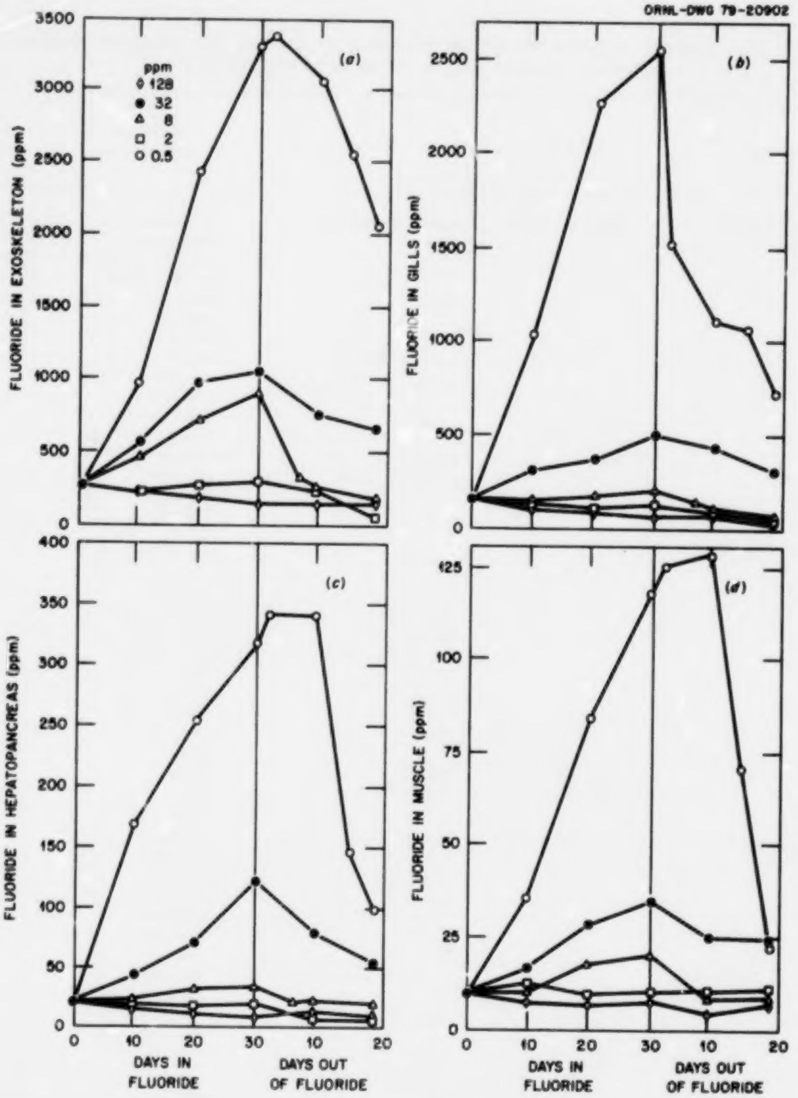

Figure 5.2. Uptake and removal of fluoride from the (*a*) exoskeleton, (*b*) gills, (*c*) hepatopancreas, and (*d*) muscle tissue of crabs exposed to 0.5, 2.0, 8.0, 32.0, and 128.0 ppm fluoride. Source: Adapted from Moore, 1971, Figures 3-6, pp. 6-8. Reprinted by permission of the publisher.

reported that fluoride excretion is accomplished through the kidneys or the respiratory epithelium in carp and rainbow trout. Such elimination is probably the main defense mechanism of these species against fluoride toxicosis. The mucous cells of carp and rainbow trout are morphologically similar to chloride-secreting cells found in other fish and may function as fluoride-secreting cells.

TABLE 5.11. FLUORIDE ANALYSIS OF TISSUES FROM AQUATIC SPECIES COLLECTED FROM LYNEMOUTH POWER STATION (ENGLAND) INFLOW FILTER AND FROM OFFSHORE SITES

Species	Location	Tissue	Month of collection	Fluoride concentration (ppm wet wt ± std deviation)[a]
Cod (*Gadus morhua*)	Inflow filter	Axial skeleton	December, 1972	42.8 ± 17.1 (3)
			January, 1973	69.3 ± 67.1 (4)
			February, 1973	20.0 (1)
			March, 1973	37.1 (2)
		Skin	December, 1972	13.3 ± 7.2 (3)
			January, 1973	21.6 ± 5.8 (4)
			February, 1973	35.6 (1)
			March, 1973	27.4 (2)
		Liver	December, 1972	2.2 ± 0.1 (3)
			January, 1973	4.6 ± 4.3 (4)
			February, 1973	0.9 (1)
			March, 1973	2.9 (2)
		Kidney	January, 1973	2.2 (2)
		Stomach wall	December, 1972	2.5 ± 1.5 (3)
			January, 1973	3.6 ± 2.1 (4)
			February, 1973	3.6 (1)
			March, 1973	3.1 (2)
		Muscle	December, 1972	1.0 ± 0.6 (3)
			January, 1973	3.4 ± 1.6 (4)
			February, 1973	0.5 (1)
			March, 1973	2.5 (2)
	Blyth Bay	Gill	May, 1973	2.1 ± 1.5 (5)
		Axial skeleton	May, 1973	23.9 ± 4.9 (5)
		Skin	May, 1973	29.6 ± 19.2 (5)
		Gonad	May, 1973	2.8 ± 1.9 (5)
		Liver	May, 1973	1.2 ± 1.9 (5)
		Kidney	May, 1973	2.1 ± 2.3 (5)
		Stomach wall	May, 1973	19.2 ± 35.2 (5)
		Muscle	May, 1973	1.7 ± 1.0 (5)
		Fat body	May, 1973	0.9 ± 0.6 (5)
		Blood	May, 1973	<0.2
Haddock (*Gadus aeglefinus*)	Inflow filter	Axial skeleton	November, 1972	18.2 (1)
			January, 1973	60.4 (1)
		Gill bar	November, 1972	100.4 (1)
		Skin	November, 1972	74.3 (1)
			January, 1973	15.5 (1)
		Liver	November, 1972	4.0 (1)
			January, 1973	1.9 (1)
		Stomach wall	November, 1972	2.0 (1)
			January, 1973	2.0 (1)
		Muscle	November, 1972	3.7 (1)
			January, 1973	2.2 (1)
	Blyth Bay	Gill	May, 1973	5.7 ± 2.9 (3)
		Axial skeleton	May, 1973	49.3 ± 24.4 (3)
		Skin	May, 1973	37.0 ± 13.12 (3)
		Gonad	May, 1973	1.5 ± 0.8 (3)
		Liver	May, 1973	0.2 ± 0.1 (3)
		Kidney	May, 1973	0.6 ± 0.7 (3)
		Stomach wall	May, 1973	3.6 ± 1.6 (3)
		Muscle	May, 1973	1.8 ± 0.7 (3)
		Fat body	May, 1973	0.5 (2)
		Blood		
Sprat (*Clupea sprattus*)	Inflow filter	Axial skeleton	October, 1972	55.1 ± 20.0 (4)
			November, 1972	52.8 ± 35.5 (3)
			December, 1972	52.5 ± 37.1 (3)
			January, 1973	52.2 ± 27.4 (3)
			March, 1973	48.5 ± 19.1 (4)
		Skin	October, 1972	22.8 ± 12.9 (4)
			November, 1972	21.3 ± 12.1 (3)
			December, 1972	10.8 ± 1.1 (3)
			January, 1973	18.7 ± 12.2 (3)
			March, 1973	14.2 ± 6.9 (4)

TABLE 5.11 (continued)

Species	Location	Tissue	Month of collection	Fluoride concentration (ppm wet wt ± std deviation)[a]
		Muscle	October, 1972	5.2 ± 0.9 (4)
			November, 1972	4.9 ± 3.4 (3)
			December, 1972	2.7 ± 1.7 (3)
			January, 1973	3.6 ± 2.2 (3)
			March, 1973	4.7 ± 1.9 (4)
Flounder (*Pleuronectes flesus*)	Inflow filter	Axial skeleton	December, 1972	19.9 (1)
			January, 1973	48.7 (1)
		Skin	December, 1972	11.6 (1)
			January, 1973	10.6 (1)
		Liver	December, 1972	1.3 (1)
			January, 1973	4.2 (1)
		Stomach wall	December, 1972	1.1 (1)
			January, 1973	16.0 (1)
		Muscle	December, 1972	1.7 (1)
			January, 1973	1.8 (1)
Lumpsucker (*Cyclopterus lumpus*)	Inflow filter	Axial skeleton	December, 1972	30.7 (1)
		Skin	December, 1972	34.3 (1)
			January, 1973	34.0 (1)
			March, 1973	12.0 (1)
		Liver	December, 1972	6.2 (1)
			January, 1973	2.2 (1)
			March, 1973	3.2 (1)
		Kidney	December, 1972	1.5 (1)
			January, 1973	3.1 (1)
			March, 1973	3.2 (1)
		Stomach wall	December, 1972	2.3 (1)
			January, 1973	4.5 (1)
			March, 1973	5.6 (1)
		Muscle	December, 1972	6.0 (1)
			January, 1973	15.1 (1)
			March, 1973	4.7 (1)
Dab (*Pleuronectes limanda*)	Inflow filter	Axial skeleton	December, 1972	51.2 (1)
			January, 1973	60.6 (1)
		Skin	December, 1972	17.8 (1)
			January, 1973	21.2 (1)
		Liver	December, 1972	1.6 (1)
			January, 1973	0.9 (1)
		Stomach wall	December, 1972	2.8 (1)
			January, 1973	2.2 (1)
		Muscle	December, 1972	1.0 (1)
			January, 1973	2.4 (1)
	Blyth Bay	Gill	May, 1973	1.9 ± 1.5 (3)
		Axial skeleton	May, 1973	99.7 ± 112.3 (3)
		Skin	May, 1973	52.2 ± 10.2 (3)
		Gonad	May, 1973	1.4 ± 0.6 (3)
		Liver	May, 1973	2.5 (2)
		Kidney		
		Stomach wall	May, 1973	4.1 ± 2.2 (3)
		Muscle	May, 1973	1.0 ± 0.2 (3)
		Fat body		
		Blood		
Shore crabs (*Carcinus maenas*)	Cresswell	Skeleton (carapace)	October, 1972	14.2 ± 6.0
			November, 1972	15.4 ± 8.3
			December, 1972	14.4 ± 4.7
			January, 1973	16.3 ± 9.7
			February, 1973	4.6 ± 1.8
			March, 1973	7.4 ± 1.5
			April, 1973	6.6 ± 7.2
		Skeleton (leg)	October, 1972	11.4 ± 3.7
			December, 1972	15.0 ± 3.9
			January, 1973	15.0 ± 3.9

TABLE 5.11 (continued)

Species	Location	Tissue	Month of collection	Fluoride concentration (ppm wet wt ± std deviation)[a]
		Gill	October, 1972	3.2 ± 2.5
			November, 1972	1.2 ± 2.3
			December, 1972	2.0 ± 0.9
			January, 1973	3.3 ± 0.9
			February, 1973	1.1 ± 0.5
			March, 1973	1.1 ± 0.7
			April, 1973	0.8 ± 0.6
		Gonad	October, 1972	1.6 ± 1.3
			November, 1972	2.5 ± 1.4
			December, 1972	2.0 ± 0.8
			January, 1973	1.0 ± 1.0
			March, 1973	0.8 ± 0.2
			April, 1973	1.7 ± 0.6
		Hepatopancreas	October, 1972	2.2 ± 1.4
			November, 1972	2.1 ± 2.1
			December, 1972	1.7 ± 0.8
			January, 1973	1.6 ± 1.51
			March, 1973	0.8 ± 0.1
			April, 1973	1.5 ± 0.7
		Muscle	October, 1972	1.5 ± 0.7
			November, 1972	3.5 ± 3.0
			December, 1972	1.0 ± 0.3
			January, 1973	2.1 ± 1.5
			February, 1973	2.0 ± 0.9
			March, 1973	1.6 ± 0.6
			April, 1973	2.7 ± 0.9
		Whole animal	October, 1972	5.0 ± 3.0
			November, 1972	4.9 ± 2.4
			December, 1972	7.2 ± 2.2
			January, 1973	8.3 ± 4.1
			February, 1973	3.2 ± 0.8
			March, 1973	3.5 ± 1.0
			April, 1973	5.4 ± 2.[illegible]
Swimming crab (*Portunus depurator*)	Inflow filter	Exoskeleton (carapace and leg)	June, 1973	11.6 ± 5.6
		Muscle	June, 1973	1.6 ± 0.6
		Whole animal	October, 1972	3.9 ± 1.3
			June, 1973	3.5 ± 0.4
Shrimp (*Crangon vulgaris*)	Inflow filter	Exoskeleton (carapace and leg)	October, 1972	9.7 ± 1.6
			June, 1973	11.5 ± 5.3
		Muscle	October, 1972	1.1 ± 0.5
			June, 1973	1.8 ± 1.9
		Whole animal	October, 1972	3.8 ± 2.8
			June, 1973	4.7 ± 2.4
Prawn (*Leander serratus*)	Inflow filter	Exoskeleton (carapace and leg)	June, 1973	11.3 ± 6.8
		Muscle	June, 1973	2.1 ± 1.2
		Whole animal	October, 1972	4.1 ± 2.0
			June, 1973	4.8 ± 1.9

[a] Number of analyses shown in parentheses.

Source: Adapted from Wright and Davison, 1975, Tables 4 and 5, pp. 5-6. Reprinted by permission of the publisher.

5.3.2 Effects

5.3.2.1 Physiological Effects — Sodium fluoride (0.01 m*M*) did not affect respiratory activity during cleavage and gastrulation of *Bufo arenarum* eggs. However, the pools of pyruvate and α-ketoglutarate were decreased

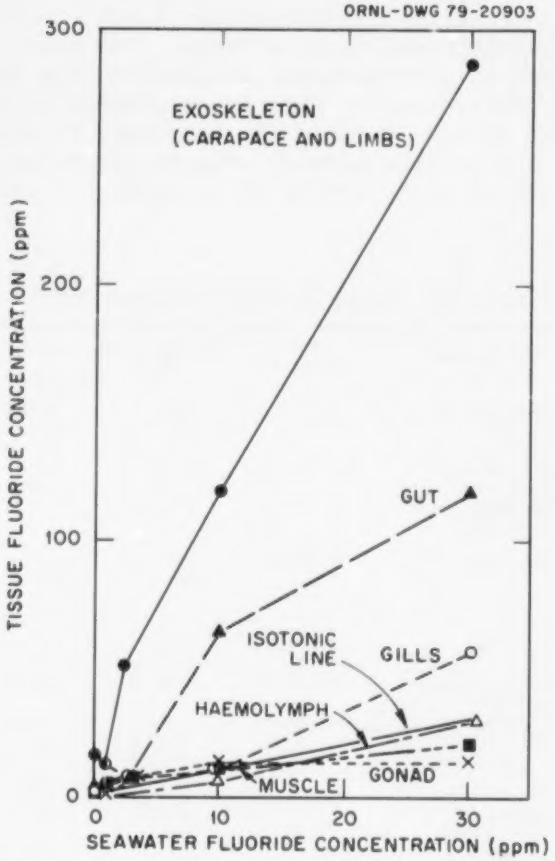

Figure 5.3. The relationship between tissue fluoride and external fluoride in *Carcinus maenas* exposed to different fluoride concentrations in seawater. Source: Adapted from Wright and Davison, 1975, Figure 1, p. 8. Reprinted by permission of the publisher.

(Barbieri and de Moreno, 1966). Decreases in pyruvate of 50% and α-ketoglutarate of 30% were detected within 4 hr after exposure. Oxygen consumption was unaffected during blastulation and gastrulation. Fluoride was believed to have penetrated the eggs and inhibited glycolysis. Exposure to sodium fluoride of annelid and clam eggs caused no inhibition of embryo development (Sawada and Rebhun, 1969).

Growth of the crab *C. sapidus* was decreased by exposure to 20 ppm fluoridated water with an average decrease of 4.7% in the growth increment at molt when compared with unexposed crabs (Moore, 1971). A decrease of 4.7% during each molt would result in a total growth reduction of 52% during the life of the crab. Since fluoride concentrations of 20 ppm have been reported in some estuaries where phosphate mining operations occur, continued exposure of crabs to such concentrations could have an adverse effect on the crabbing industry.

5.3.2.2 Toxicity — Fluoride concentrations above certain levels in aquatic systems have profound toxic effects. The response of aquatic organisms is related to environmental acclimatization and species of organism. The concentrations of fluoride in otherwise unpolluted water have no detrimental effects (Becker and Thatcher, 1973). Concentrations of 1.5 ppm appear not to have harmful effects on aquatic organisms. Table 5.12 presents a list of toxic levels of fluoride for various aquatic species.

TABLE 5.12. TOXICITY OF FLUORIDES TO AQUATIC SPECIES

Chemical compound	Test organism	Test conditions[a]	Concentration (ppm)	Remarks
Sodium fluoride	*Carassius auratus*	SB, FW, LS	1000	Killed in 60-102 hr, hard water
			1000	Killed in 12-29 hr, soft water
			100	No kill in 4 days, hard water
	Polycelis nigra (planaria)	SB, FW, LS	0.0011 *M*	Toxic threshold, survives 48 hr; pH 6.6, 14-18°C
	Daphnia magna (cladocera)	SB, FW, LS	504	"Toxicity threshold" or dose to just immobilize in 48 hr; Lake Erie water, 25°C
	Gambusia affinis	SB, FW, LS	1240	24-hr TL_m, acute
			925	48- and 96-hr TL_m, acute; all data at pH 7.1-8.1, 21-24°C, turbid water
Sodium fluoride (F)	*Salmo gairdneri*	SB, FW, LS	5.9-7.5	48-hr TL_m, acute; 7.2°C
			2.6-6.0	48-hr TL_m, acute; 12.8°C
	Homarus americanus (lobster)	SB, FW, LS	0.9-4.5	Nontoxic in 10 days; 2°C
	Salmo gairdneri	CB, FW, LS	2.7-4.7	20-day TL_m; 12.8°C; fingerlings
			222-273	424-hr TL_m; 7.8°C; eggs
			242-261	214-hr TL_m; 12.8°C; eggs
			237-261	167-hr TL_m; 15.6°C; eggs
			61-85.3	825-hr TL_m; 12.8°C; fry
	Cyprinus carpio		75-91	20-day TL_m; 18.3-23.9°C
	Gambusia affinis		500	50% kill in 14 hr and 100% kill in 19 hr; anion normality 0.026
			500	50% kill in 30 min and 100% kill in 1 hr; anion normality increased to 0.26
	Carassius auratus	SB, FW, LS	120	Killed in 4 days
	Tinca vulgaris	FW, LS	678	Lethal dose
	Escherichia coli (bacteria)		180	Toxic threshold, 4 days; 27°C
			45	Toxic threshold, 4 days; 24°C
	Daphnia magna (cladocera)		270	Toxic threshold, 48 days; 23°C
	Salmo gairdneri	SB, FW, LS	5.9-7.5	10-day TL_m; 7.2°C
			2.6-6.0	10-day TL_m; 12.8°C
			2.3-7.3	10-day TL_m; 18.3°C; Ca and Mg ions held at low values
	Microregma heterostoma (protozoa)	SB, FW, LS	226	Toxic threshold, 28 hr; 27°C
Fluoride (F)	*Salmo gairdneri*	SB, FW, LS	250	Total kill in 21 days, hard water
			150	90% kill in 21 days, hard water
			100	No toxic effect in 21 days, hard water
			113	Total kill in 21 days, soft water
			75	No toxic effect in 21 days, soft water
Fluoride	Minnows	FW	7.7	Not harmed in 1 hr
	Carassius auratus		100	Survived over 4 days
			1000	Killed in 12-29 hr, soft water
			1000	Killed in 60-102 hr, hard water

[a] SB — static bioassay; CB — constant-flow bioassay; FW — freshwater; SW — sea (salt) water; LS — lab study.

Source: Adapted from Becker and Thatcher, 1973, Table R, p. R2. Data collected from several sources.

Crustaceans are able to tolerate high levels of fluoride in seawater (Wright and Davison, 1975). The effects of various fluoride levels on three species of crab, *Carcinus maenas*, *Cancer pagurus*, and *Portunus depurator*, are shown in Table 5.13. The mussel *Mytilus edulis* also showed toxic effects when exposed to water containing 30 ppm fluoride for two or three weeks. Mussels exposed to 10 ppm fluoride died after five-week exposures. Evidence from this study shows that the aquatic animals most sensitive to fluoride appear to be filter-feeding molluscs.

Frogs (*Rana pipiens*) exposed to sodium fluoride in water at concentrations ranging from 5 to 50 ppm showed few external signs of toxicosis but had a definite decrease in total red and white cell counts (Kaplan, Yee, and Glaczenski, 1964). Changes in the internal organs, engorgement of blood vessels, and local hemorrhages in the stomach and intestines occurred at 50 ppm fluoride. Death occurred at 28 days in 50 ppm fluoride, and as the fluoride concentrations increased, the survival time shortened and the percentage of deaths increased. Death followed after a 2-hr immersion in 3.5 parts-per-thousand (ppt) fluoride, after 15 hr in 2.8 ppt, 18 hr in 2.5 ppt, 50 hr in 2.2 ppt, 72 hr in 1.5 ppt, and 72 hr in 1 ppt. Fluoride entered the frogs by ingestion and by penetration through the skin. Kaplan, Yee, and Glaczenski (1964) also discussed experiments in which injection of sodium fluoride in concentrations as high as 1000 ppm (no data given on total amount of solution injected) into the dorsal lymph sacs produced only a slight drop in red and white cell counts. Blood clotting time was not changed by injection of sodium fluoride, whereas clotting time was delayed when frogs were immersed in solutions containing concentrations of fluoride as low as 30 ppm. This difference in buildup of blood levels may partially explain the differences in fluoride toxicosis following different means of exposure.

Fish exposed to sodium fluoride become apathetic, lose weight, have periods of violent movement, and wander aimlessly. Finally, there is a loss of equilibrium accompanied by tetany and death. Mucus secretion increases, accompanied by proliferation of mucus-producing cells in the respiratory and integumentary epithelium (Neuhold and Sigler, 1960). Alger (as cited in Sigler and Neuhold, 1972) reported a decrease in total serum protein in trout and carp during fluoride intoxication. Serum alkaline phosphatase activity and plasma magnesium levels increased and calcium decreased with exposure to solutions containing increasing fluoride concentrations. Fish size also affected toxicity; the larger the fish, the more resistant they were to a given level of fluoride.

Symptoms of acute fluoride intoxication in rainbow trout and carp were lethargy, erratic movement, and death when partial or complete muscle contraction occurred (Neuhold and Sigler, 1960). Rainbow trout embryos died within the egg and displayed symptoms similar to those of adults. Hatching time of eggs decreased with exposure to increasing fluoride concentrations; however, the earlier hatching embryos were not as developed as those hatching later. Variables such as size of fish, temperature of the medium, and calcium and chloride concentration of the medium affected the lethal doses and sensitivity of the fish. As the fish size increased, the length of time before mortality increased at a given fluoride level;

TABLE 5.13. THE EFFECT OF INCREASED FLUORIDE (AS SODIUM FLUORIDE) IN ARTIFICIAL SEAWATER ON VARIOUS MARINE, INTERTIDAL ANIMALS OF THE NORTHUMBRIAN COAST

Species	Number of animals used at each fluoride concentration	Fluoride-free	1 ppm fluoride	2-4 ppm fluoride	10 ppm fluoride	30 ppm fluoride
Swimming crab (*Portunus depurator*)	2	Alive after 90 days	One dead after 60 days, other alive at 90 days	Alive after 90 days	Alive but unhealthy after 90 days	Alive after 90 days
Edible crab (*Cancer pagurus*)	1	Alive after 90 days	Alive after 90 days	Alive after 90 days	Alive after 90 days	Alive after 90 days
Shore crab (*Carcinus maenas*)	4	Alive after 90 days	Alive after 90 days	Alive after 90 days	Alive after 90 days	Alive after 90 days
Edible mussel[a] (*Mytilus edulis*)	4	Alive at 42 days	Two dead after 10 days (lack of O_2 probable cause as aerator stopped) Two still alive after 42 days	Alive at 42 days	Three dead after 30 days, fourth dead after 36 days	Three dead after 14 days, fourth dead at 21 days

[a] In a preliminary experiment at room temperature (groups of four animals), all animals survived 0, 1, and 2.44 ppm for two weeks; in 10 ppm fluoride, two dead at two weeks; in 30 ppm fluoride, all dead in ten days.

Source: Adapted from Wright and Davison, 1975, Table 9, p. 10. Reprinted by permission of the publisher.

however, the LD_{50} did not change. An increase in temperature altered the sensitivity from 2.02 ppm fluoride at 7°C to 10.81 ppm at 16°C. The LD_{50} increased from 246 ppm fluoride at 7°C to 251 ppm at 16°C. Higher than normal concentrations of calcium in the medium increased resistance of fish to fluoride. The toxicity of fluoride to mosquitofish (*Gambusia affinis*) increased when the total anion normality was increased by adding sodium chloride to the medium. Mortality time for 50% of the fish at 500 ppm fluoride was 14 hr; increasing anion normality from 0.026 to 0.26 by adding chloride decreased the mortality time for 50% of the fish to 30 min. Calcium deficiency induced by high fluoride levels seemed to cause hypertrophy of the ultimobranchial gland. Fluoride forms a stable mineral complex with calcium, making calcium less available for mobilization from the bone. The uptake of fluoride by the bone is a defense mechanism against fluoride toxicosis. This mechanism works by eliminating fluoride from body circulation.

5.4 BIRDS

5.4.1 Metabolism

5.4.1.1 Uptake — Fluoride in poultry is primarily derived from ingestion of food and water containing fluorides (Cass, 1961). Minimal quantities can enter the body through the respiratory tract. These fluorides are in either a gaseous or particulate form. Systemic absorption of inorganic fluorides through the skin is negligible.

5.4.1.2 Distribution and Accumulation — Kay, Tourangeau, and Gordon (1975*a*) determined the fluoride levels in indigenous animals collected from ecosystems uncontaminated by industrial fluoride. Femur fluoride levels found in birds are given in Table 5.14. Table 5.15 presents the natural or "background" fluoride levels found in birds in a nonindustrialized area in New Zealand (Stewart et al., 1974).

TABLE 5.14. FEMUR FLUORIDE LEVELS IN BIRDS COLLECTED FROM UNCONTAMINATED ECOSYSTEMS

Species	Sample size	Mean fluoride level (ppm dry wt)
Blackbilled magpie (*Pica pica*)	4	535.8
Blue grouse (*Dendragapus obscurus*)	3	321.3
Ruffed grouse (*Bonasa umbellus*)	5	127.6
Sage grouse (*Centrocercus urophasianus*)	1	216.0
Sharptail grouse (*Pedioecetes phasianellus*)	1	97.0
Spruce grouse (*Canachites canadensis*)	2	176.5

Source: Adapted from Kay, Tourangeau, and Gordon, 1975*a*, Table 1, p. 127. Reprinted by permission of the publisher.

TABLE 5.15. BONE AND GIZZARD FLUORIDE CONCENTRATIONS IN BIRDS IN AN UNCONTAMINATED AREA OF NEW ZEALAND

Species	Number of samples	Diet	Fluoride concentration (ppm)			
			Bone[a]		Gizzard contents[b]	
			Mean	Range	Mean	Individual values
Red-billed gull (*Larus novaehollandiae scopulinus*)	16	Mainly macroplankton (predominantly euphausid crustacea), small forage fish, some beach scavenging, some insects and worms from agricultural land	4003	1058-8050	28	9.4, 24, 50
White-faced heron (*Ardea novaehollandiae*)	3	Small fish, tadpoles, frogs, soft-shelled crabs, freshwater crayfish, and aquatic insects	2208	1006-3264	35	13, 30, 62
Mallard duck (*Anas platyrhynchos*)	11	Aquatic plants, freshwater molluscs, soft-bodied invertebrates (e.g., worms and slugs)	1902	430-5440	13	4, 9.2, 28
Black-backed gull (*Larus dominicanus*)	16	Mollusks, offal, and garbage	1907	754-3140	144	2.9, 24, 405
Harrier hawk (*Circus approximans*)	14	Large insects (e.g., grasshoppers), young fish, mice, lizards, frogs, and carrion (hares, opossums, and hedgehogs)	1445	379-4775	3.5	1.9, 5.2
Pied oyster-catcher (*Haematopus ostralegus finschi*)	14	Shellfish, marine worms, grass grubs, and insect larvae	1243	393-1965	6	1.9, 4.1, 12
Hedge sparrow (*Prunella modularis*)	1	Insects, buds, and seeds	1021			
Starling (*Sturnus vulgaris*)	14	Grass grubs, birds' eggs, nectar, and scavenges in seaweed	703	157-1390	3.1	2.0, 3.6, 3.7
Pukeko (*Porphyrio melanotus*)	16	Main diet consists of stems, leaves, seedheads of plants, birds' eggs, and insects	489	143-1400	1.7	1.0, 1.7, 2.6

[a] Ash-weight basis.
[b] Fresh-weight basis.

Source: Adapted from Stewart et al., 1974, Table 2, p. 108. Reprinted by permission of the publisher.

Fluoride levels in seabirds from the coast of Great Britain indicate that higher fluoride levels occur in the skeletal tissue than in the soft tissues (Table 5.16). The apparently high bone fluoride levels are well within the range found in other terrestrial vertebrates (Wright and Davison, 1974).

TABLE 5.16. TISSUE FLUORIDE LEVELS OF SEABIRDS FROM THE COAST OF GREAT BRITAIN (ppm wet wt)

Tissue	Guillemots				Greater black-backed gulls, Newbiggin, October 1972
	Newbiggin, November 1972	Newbiggin, August 1972	Whitley Bay, January 1972	Mousehole, Cornwall, January 1972	
Liver	<0.2	<0.2	1.61	0.95	0.42
Heart	0.25			0.88	
Crop	0.2	12.0			0.61
Kidney	1.12	0.3	0.43		0.20
Muscle (pectoralis major)	1.67		1.99	1.00	0.26
Tibia	40		750		
Skull bone					0.72
Skin		1.5			0.81
Feathers		1.5			0.60
Lung			1.18		

Source: Adapted from Wright and Davison, 1974, Table 3, p. 120. Reprinted by permission of the publisher.

Roosters receiving a 200-mg/kg dose of sodium fluoride twice a day for 24 hr showed the highest percent of nonskeletal fluoride ion accumulation in the liver, heart, and feathers (Slesinger and Tusl, 1966). However, in both the control and experimental roosters the highest fluoride levels occurred in bones.

Cass (1961) reported that from 0 to 0.02% of a fluoride dose injected into the bloodstream of laying hens was recovered from developing eggs. The less mature egg yolks had the highest fluoride concentration, while in mature eggs, the fluoride level in the albumin was higher than in the yolk.

Anderson et al. (1955) fed turkeys various levels of sodium fluoride and found that the fluoride concentration in bones increased as the fluoride level in the feed increased. The concentration of fluoride in bones also increased as the length of time the feed was administered increased (Table 5.17). Fluoride levels in soft tissues increased with the fluoride level in the feed, but did not increase with time.

5.4.2 Effects

5.4.2.1 Physiological Effects — Interaction between fluoride and magnesium in chicks affects both skeletal and body growth. A fluoride supplemental level of 0.08% (added as sodium fluoride to diets) reduced growth rate but

TABLE 5.17. FLUORIDE LEVELS IN TISSUES OF TURKEYS FED VARIOUS AMOUNTS OF SODIUM FLUORIDE

Tissue	Initial fluoride concentration (ppm)[a]	Treatment time (weeks)	Fluoride level in tissues at various amounts of fluoride in ration (ppm)					
			43 ppm	100 ppm	200 ppm	400 ppm	800 ppm	1,600 ppm
Sternum	[b]	8	450	740	1,500	4,050	8,000	12,300
		16	383	1,268	1,975	5,650	12,500	14,700
Femur	[b]	8	390	770	1,660	4,050	8,550	12,000
		16	360	1,210	1,775	5,400	14,875	14,900
Breast flesh	1.2	8	1.3	2.2	2.7	8.2	5.6	30.9
		16	2.2	1.5	2.3	2.7	7.5	24.6
Thigh flesh	1.5	8	1.9	2.3	3.9	2.8	7.8	11.8
		16	1.9	1.6	1.5	2.0	6.1	9.4
Liver	1.9	8	2.7	2.4	4.7	5.6	9.1	15.6
		16	1.6	1.8	6.3	2.5	3.8	11.3
Kidney	2.6	8	5.6	10.7	5.9	9.3	13.5	39.0
		16	6.5	8.2	9.0	9.7	16.2	36.1

[a] All values are on a dry basis except those from bones, which are on a dry, fat-free basis.
[b] Samples lost.

Source: Adapted from Anderson et al., 1955, Table 4, p. 1151. Reprinted by permission of the publisher.

did not affect bone ash, bone calcium or phosphorus, or plasma magnesium or inorganic phosphorus (Gardiner, Rogler, and Parker, 1961). A combination of 0.08% supplemental fluoride and 0.25% supplemental magnesium caused a greater reduction in growth than fluoride alone. The combination of fluoride and magnesium also produced leg weakness, reduced bone ash, and caused a smaller calcium and phosphorus bone content. Along with reduced growth rate and leg weakness, Griffith, Parker, and Rogler (1964) found that the addition of supplemental fluoride and magnesium to diets of chicks reduced the citric acid content of bones by 64% when compared with bones of chicks maintained on a basal diet. Fluoride supplementation alone reduced bone citrate by an average of 32%. Later, Rogler and Parker (1972) reported that excess amounts of calcium improved the growth rate of chicks fed fluoride alone or fluoride plus magnesium; however, the growth rate was inferior to that of the controls. In chicks fed high fluoride diets, bone ash was increased and the fluoride content of plasma and bone was reduced when excess calcium was added to the diets. These alleviating effects of calcium result from reduced absorption of fluoride and magnesium.

Some fluoride compounds inhibit specific enzymes in hens. Sulfonyl fluoride progressively inhibited "neurotoxic esterase" by attaching a substituent covalently at the active site (Johnson, 1970). Inhibition of the enzyme by phenylmethanesulfonyl fluoride did not induce neurotoxic effects; prior inhibition by sulfonyl fluorides in vivo prevented neurotoxic effects of organophosphorus compounds. Dietary fluoride had little or no effect on several enzyme systems of chicks (Weber, Doberenz, and Reid, 1969). Cytochrome oxidase activity in heart muscle was elevated at 500 ppm fluoride but not at 1000 ppm fluoride. Increasing dietary fluoride levels increased plasma alkaline phosphatase activity (Table 5.18).

TABLE 5.18. THE EFFECT OF DIETARY FLUORIDE ON ENZYME SYSTEMS OF CHICKS

Enzyme system	Tissue	Enzyme activity[a]		
		0 ppm fluoride	500 ppm fluoride	1000 ppm fluoride
Lactic dehydrogenase[b]	Liver	669 a		734 a
	Kidney	271 a		272 a
Cytochrome oxidase[c]	Liver	42.7 a	40.0 a	47.9 a
	Kidney	31.5 a	32.6 a	29.6 a
	Heart	36.2 a	48.8 b	42.9 ab
Succinic dehydrogenase[c]	Liver	16.6 a	17.4 a	18.4 a
	Kidney	19.0 a	19.0 a	18.8 a
Isocitric dehydrogenase[c]	Liver	20.0 a	15.6 a	15.8 a
Alkaline phosphatase[d]	Plasma	27.8 a	34.1 ab	40.1 b

[a] Means not having common letters are significantly different at the 0.05 level of probability.

[b] Enzyme activity is given as delta oxygen demand per milligram per minute.

[c] Enzyme activity is given as delta oxygen demand per milligram per hour.

[d] Enzyme activity is given as sigma units per milliliter.

Source: Adapted from Weber, Doberenz, and Reid, 1969, Table 4, p. 233. Reprinted by permission of the publisher.

High levels of fluoride affected the tibia bone development in chickens (Hicks and Ramp, 1975; Smith et al., 1970). Tibia bone ash was significantly reduced, whereas fluoride content of fat-free dried bones increased. Hicks and Ramp (1975) reported that in vitro fluoride either increased or decreased bone mineralization in embryonic chick tibia at low calcium and phosphorus concentrations, respectively, indicating that the effects are dependent on cellular control of calcium and phosphorus compounds in bone extracellular fluid. From these data, it was suggested that fluoride plays a role in the rate of mineralization in growing bones.

5.4.2.2 Toxic Effects — High fluoride ingestion by birds can result in reduced growth rate, leg weakness, and bone lesions. Tolerance to fluorides varies among bird species and among individuals of the same species. Birds are usually more resistant than mammals to toxic effects of fluoride ingestion (Shupe, 1969).

5.4.2.2.1 Acute toxicity — Typical symptoms of acute toxicity are a reduction or loss of appetite, local or general congestion, and submucosal hemorrhages of the gastrointestinal tract (Cass, 1961). Feeding chickens for ten days on a diet containing 6786 ppm fluoride produced acute responses; a one-time dose of 27.1 ppm $BaSiF_6$ on grain was lethal. Roosters receiving a 200-mg/kg dose of sodium fluoride twice a day for 24 hr developed gastroenteritis with edema of the mucosa of the stomach and upper bowels, subcutaneous edema, hepatomegaly, and atrophy of the pancreas (Slesinger and Tusl, 1966).

5.4.2.2.2 Chronic toxicity — Chronic fluorosis in birds can be difficult to diagnose and is dependent on several factors. It is necessary to establish the presence of characteristic lesions, a history of exposure at the proper time to high levels of fluoride, and analytical evidence that bone contains fluoride concentrations consistently associated with lesions of chronic fluorosis before a definite diagnosis can be made (Cass, 1961).

In birds, chronic fluoride toxicosis develops slowly and primarily involves gross and microscopic changes in bone (Cass, 1961). If elevated fluoride intake persists, the bird's general health progressively deteriorates. Lameness may develop, and there commonly is a loss of appetite.

High levels of fluoride ingestion often induce a reduced growth rate in poultry. Weight gains by male turkeys were decreased by the addition of 200 ppm fluoride in the ration (Anderson et al., 1955). A critical fluoride level between 200 and 400 ppm exists for growing turkeys. Between these levels there were (1) decreases in weight gain for male and female turkeys, (2) decreases in food consumption, (3) decreases in feed efficiency, and (4) gross changes in the intestinal tract.

Fluoride added to feed at a rate of 1000 ppm reduced the weight gain of chicks, while a combination of 1000 ppm fluoride and 10,000 ppm bromide caused further weight reductions (Doberenz et al., 1965). A diet containing 2000 ppm fluoride plus 10,000 ppm bromide caused 100% mortality within three weeks. Fluoride alone produced 25% mortality by four weeks of age. Weber, Doberenz, and Reid (1969) also reported a growth reduction in chicks fed 500 ppm fluoride; a 21% reduction occurred at 1000 ppm fluoride. Along with a reduction in body-weight gain, Gardiner, Winchell, and Hironaka (1968) found that an 0.08% fluoride diet supplement caused a decrease in feed consumption and metabolizable energy from the feed. The total energy needed per unit weight gain was increased. The difference in growth rate between control and experimental chicks was due to a 51.4% decrease in feed consumption, a 13.3% decrease in metabolizable energy from the feed, and a 33.3% lower efficiency of energy metabolism.

Possibly one reason for the decrease in feed consumption of chickens fed high levels of fluoride is the changes that take place in the proventriculus. Dietary fluoride at 0.08% and 0.10% increased the size and weight of the proventriculus (Gardiner et al., 1959). Cellular changes were most pronounced on the gland surface and included hypertrophy and hyperplasia of the columnar epithelium and a thickening of the tunica propria.

5.4.2.2.3 Teratogenicity — Injection of 5-fluorouracil, 5-fluoro-2'-deoxyuridine, or 5-fluoroorotic acid into the yolk sac during the first four days of development of the chicken embryo produced specific developmental anomalies that varied with the stage of development at the time of injection and the compound injected (Kury and Craig, 1966). Skeletal malformations included the absence, shortening, curvature, and/or fusion of bones. Microphthalmia, defective eyelids, retardation of growth of extremities, and abnormal bill development were common malformations induced by the three fluorinated pyrimidine compounds used.

5.4.2.2.4 Tolerance to fluoride — Poultry are more resistant to fluoride toxicity than most other animals. They also usually ingest smaller amounts of fluoride than most animals because of the low fluoride content of grains (National Academy of Sciences, 1971). Resistance to fluorides in the young chick is due to the lack of adverse effects from a high serum fluoride content (Suttie, Phillips, and Faltin, 1964). Growing chicks can tolerate up to 300 ppm fluoride in their diet (National Academy of Sciences, 1974).

Laying hens have a higher tolerance to fluoride than chicks. Feeding chickens 5000 ppm fluoride (based on body weight) one time failed to produce any symptoms of fluoride toxicity (Cass, 1961). Turkeys have a tolerance level of 200 ppm dietary fluoride, above which significant decreases in growth rate occur (Anderson et al., 1955). Vohra (1973) reported that 50 ppm fluoride, as sodium fluoride, in the drinking water of Japanese quail did not affect body weight, tibia weight, bone ash, or eggshell thickness. Fluoride concentrations up to 200 ppm were tolerated by the quail.

5.5 DOMESTIC AND WILD MAMMALS

5.5.1 Metabolism

The metabolism of fluorine-containing compounds by domestic and wild mammals is similar to that of humans, and much of the data presented in Section 6 are applicable to mammals discussed in this section. However, metabolism data dealing directly with these mammals, particularly livestock, are presented in this section.

5.5.1.1 Uptake and Absorption

5.5.1.1.1 Routes of entry — Fluoride in the body of mammals is derived mainly from the ingestion of fluoride-containing food and water (Cass, 1961). Usually only small quantities of fluoride in gaseous or particulate form enter the body through the respiratory tract. Absorption of inorganic fluorides through the skin is insignificant.

The principal sources of excessive fluoride to livestock are ingestion of high-fluoride vegetation in areas with a fluoride pollution problem (National Academy of Sciences, 1971) and the inclusion of raw rock phosphate in the diet (Dale and Crampton, 1955). Other sources of fluoride for mammals are waters containing high concentrations of fluoride and the ingestion of soils high in fluorides.

5.5.1.1.2 Absorption — The absorption of soluble fluorides from the gastrointestinal tract is rapid and almost complete (Underwood, 1971). This absorption appears to be passive and does not involve active transport (National Academy of Sciences, 1974). Perkinson et al. (1955) reported that in sheep and cattle, absorption took place rapidly from the rumen. Approximately 1% of an ingested dose of fluoride represented the peak blood value, which occurred at 2- and 5-hr postingestion in sheep and cows respectively.

The gastrointestinal absorption of fluorides is greatly influenced by the chemical form of the fluoride and the dietary consumption. Generally, the less soluble a fluoride compound is, the slower the rate of absorption (Largent, 1961; National Academy of Sciences, 1974). However, small amounts of relatively insoluble fluorides ingested in solution can be absorbed almost as well as the more soluble forms (Underwood, 1971). Large amounts (1% to 2%) of certain ions in the diet (e.g., aluminum, calcium, and magnesium) form less soluble complexes with fluoride, decreasing the amount of absorbable fluoride (National Academy of Sciences, 1974; Shupe, Olson, and Sharma, 1972).

5.5.1.2 Accumulation and Distribution

5.5.1.2.1 Accumulation — Natural fluoride levels in the skeletons of small mammals, cattle, and sheep in the Bluff area of New Zealand were determined before the operation of an aluminum smelter (Stewart et al., 1974). Bone fluoride levels ranged from 20 to 1500 ppm, bone-ash basis (Table 5.19). Variation of fluoride levels within species was mainly due to age differences, since specimens were collected randomly without regard to age. Fluoride levels in lambs and calves were lower than in adults. Bone fluoride levels in wildlife seemed to be a function of amount and duration of exposure. Differences in fluoride-tissue concentrations between species were probably due to differences in dietary exposure.

TABLE 5.19. FLUORIDE CONCENTRATIONS IN BONES OF WILD AND DOMESTIC ANIMALS

Sample	Number of samples	Bone fluorine concentrations (ppm)[a]	
		Mean	Range
Opossum (*Trichosurus vulpecula*)	2	247	216-278
Rabbit (*Oryctolagus cuniculus*)	1	184	
Cattle			
Jawbone	1	1207	
Tail			
Adult	105	643	229-1469
Calf	32	245	19-944
Sheep			
Jawbone	17	735	352-1178
Tail			
Adult	11	327	91-990
Lamb	36	202	42-669

[a] Bone-ash basis.

Source: Adapted from Stewart et al., 1974, Table 4, p. 110. Reprinted by permission of the publisher.

The results of a study to determine fluoride levels in animals from an uncontaminated ecosystem are given in Table 5.20. Animals collected from areas near human habitation or those maintained on a commercial laboratory ration had higher fluoride levels than the animals collected from a natural ecosystem (Kay, Tourangeau, and Gordon, 1975*a*). Redtail chipmunks caught wild and fed a laboratory ration for three months had over 1000 ppm fluoride (dry, fat-free basis) in the femurs, whereas chipmunks not fed the ration had only 77 ppm fluoride. Predatory mammals appeared to have higher levels of femur fluoride than herbivorous animals. The higher fluoride concentrations in predatory animals were thought to result from consumption of prey skeletons.

TABLE 5.20. FLUORIDE LEVELS IN FEMURS OF WILD ANIMALS

Species	Sample size	Mean fluoride level in femur (ppm)[a]
Beaver (*Castor canadensis*)	3	148.7
Black-tailed jackrabbit (*Lepus californicus*)	1	122.0
Black-tailed prairie dog (*Cynomys ludovicianus*)	1	120.0
Chipmunk (*Eutamias* sp.)	19	103.1
Columbian ground squirrel (*Citellus columbianus*)	23	112.5
Coyote (*Canis latrans*)	1	321.0
Mandible	1	257.0
Deer mouse (*Peromyscus maniculatus*)	70	143.8
Golden mantled squirrel (*Citellus lateralis*)	2	67.0
Long-tailed weasel (*Mustela frenata*)	2	279.0
Lynx (*Lynx canadensis*)	1	176.0
Masked shrew (*Sorex cinereus*)	1	589.0
Meadow vole		
Microtus pennsylvanicus	3	139.0
Microtus montanus	2	133.0
Mink (*Mustela vison*)	2	499.5
Moose (*Alces alces*) (mandible 5.5 years old)	1	248.0
Mountain cottontail (*Sylvilagus nuttalli*)	4	167.5
Muskrat (*Ondatra zibethicus*)	11	266.4
Northern flying squirrel (*Glaucomys sabrinus*)	6	141.8
Northern water shrew (*Sorex palustris*)	1	340.0
Ord kangaroo rat (*Dipodomys ordi*)	2	440.5
Porcupine (*Erethizon dorsatum*)	6	161.0
Red squirrel (*Tamiasciurus hudsonicus*)	9	151.9
Red-backed vole (*Clethrionomys gapperi*)	5	258.0
Red-tailed chipmunk (*Eutamias ruficaudus*)	2	77.5
Richardson ground squirrel (*Citellus richardsoni*)	1	64.0
Short-tailed weasel (*Mustela erminea*)	5	363.6
Snowshoe hare (*Lepus americanus*)	4	138.2
Stone sheep (*Ovis dalli stonei*) (mandible 8.5 years old)	1	380.0
Vagrant shrew (*Sorex vagrans*)	5	474.8
Western big-eared bat (*Plecotus townsendi*)	1	78.0
White-tailed jackrabbit (*Lepus townsendi*)	14	258.6
Yellow pine chipmunk (*Eutamias amoenus*)	1	67.0

[a] Dry, defatted weight.

Source: Adapted from Kay, Tourangeau, and Gordon, 1975*a*, Table 1, p. 127. Reprinted by permission of the publisher.

5.5.1.2.2 *Transport* — Absorbed fluoride is transported rapidly by the blood throughout the body as the fluoride ion (Underwood, 1971). Fluorine ions easily cross cell membranes, including the red blood cell. Plasma fluoride concentration is usually twice the level in red blood cells and is approximately 75% of the total blood fluoride concentration (National Academy of Sciences, 1974). Fluoride leaves the blood rapidly and is distributed to various body tissues.

5.5.1.2.3 *Distribution* — Fluorides are distributed throughout the body and are selectively accumulated in skeletal and mineralizing dental tissues (Shupe, Olson, and Sharma, 1972). Throughout the life of the animal, fluorides are deposited in bones; approximately 98% of the total fluoride body burden accumulates in mineralized tissues. Fluoride deposition, being related to tissue metabolism, occurs more readily in forming and mineralizing teeth and bones. The concentration of fluoride ions in soft tissues approximates that of the plasma.

5.5.1.2.3.1 *Blood* — Fluorides occur in intracellular fluid (National Academy of Sciences, 1974). Erythrocytes contain a fluoride level that is 40% to 50% of that in plasma. Five percent of the plasma fluoride is bound to protein; the compounds that bind fluoride have a lower molecular weight than albumin. Fluoride concentrations in plasma are controlled by regulatory mechanisms involving skeletal and renal tissues. The concentration of plasma fluoride in animals not exposed to higher than background fluoride levels is very low, usually 0.1 to 0.2 ppm (Underwood, 1971).

Elevated plasma fluoride concentrations occur in mammals during periods of high fluoride intake. Plasma fluoride levels change rapidly in response to changes in fluoride intake levels. Suttie, Carlson, and Faltin (1972) alternated six-month periods of high- and low-fluoride intake levels in dairy cattle and found that the plasma levels rose or fell accordingly within a week. Also affecting plasma fluoride concentration is the method of fluoride administration. Perkinson et al. (1955) administered ^{18}F to lambs and cows. Figures 5.4 and 5.5 illustrate the ^{18}F levels in the blood of lambs over time. Fluorine-18 given intravenously produced a higher initial blood fluorine level than a dose given orally, and the rate of removal from the blood was more rapid for the intravenous dose.

A regular fluctuation in plasma fluoride has been reported to occur following oral administration of a single dose per day or during continuous intrarumenal infusion (Simon and Suttie, 1968). When sheep were given 2 ppm fluoride (based on body weight), a rapid increase in plasma fluoride occurred, peaking 3 hr after the dose and then decreasing to preingestion levels by 12 hr after the dose (Figure 5.6).

Cows fed 1.2, 1.4, or 2.0 ppm fluoride (based on body weight) for four years followed by two years without added fluoride had 0.12 ppm, 0.36 and 0.53 ppm, and 0.48 and 1.12 ppm fluoride plasma levels respectively (Phillips, Suttie, and Zebrowski, 1963). Bell, Merriman, and Greenwood (1961) reported high levels of ^{18}F in blood and rapid removal rate following intravenous injection of tracer levels in cattle. Two minutes following injection, 53% of the dose remained in the blood; after 240

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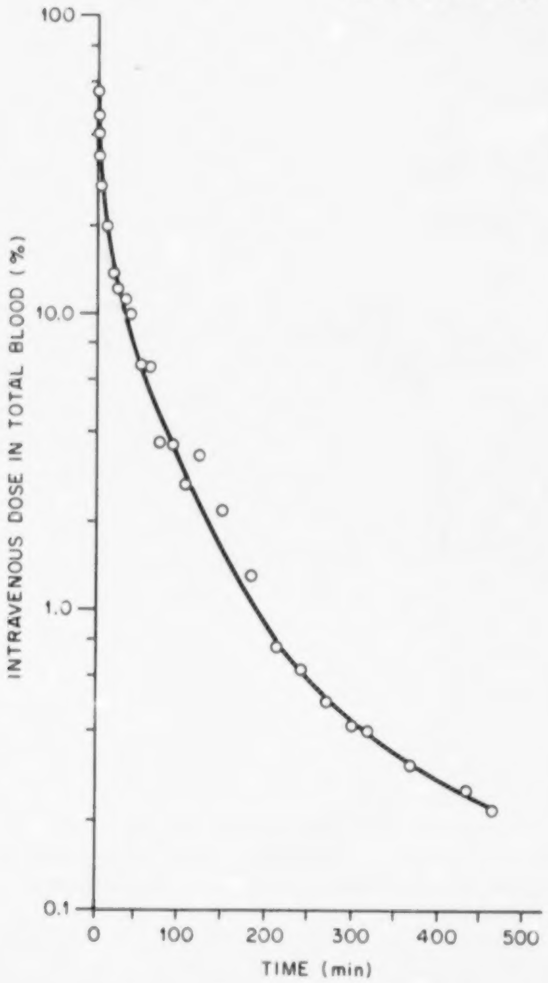

Figure 5.4. Fluorine-18 concentration in blood of lambs following intravenous administration. Source: Adapted from Perkinson et al., 1955, Figure 1, p. 384. Reprinted by permission of the publisher.

minutes, 4% of the dose was in the blood (Figure 5.7). Bogdanov and Vlasova (1973) reported 0.7 ppm fluoride in plasma of cows fed phosphates containing 0.2% to 0.4% fluoride.

5.5.1.2.3.2 Bones and teeth — Fluoride accumulates in bone and is incorporated into hydroxyapatite, forming fluorapatite (National Academy of Sciences, 1974). Following incorporation of fluoride ions into the apatite of bone, the ions cannot be removed without resorption of the unit crystalline structure of the mineral phase. Fluoride levels normally increase with the age of the animal and in proportion to duration, rate, amount, and form of the fluoride intake (Underwood, 1971). The rate of

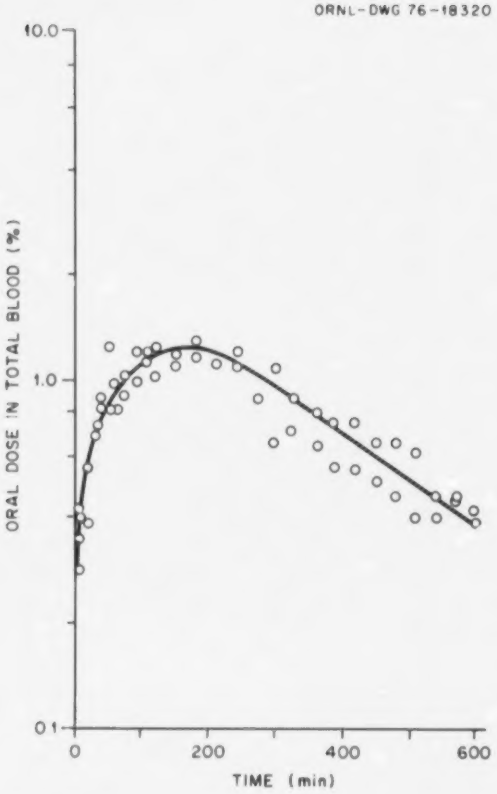

Figure 5.5. Fluorine-18 concentration in blood of lambs following oral administration. Source: Adapted from Perkinson et al., 1955, Figure 2, p. 384. Reprinted by permission of the publisher.

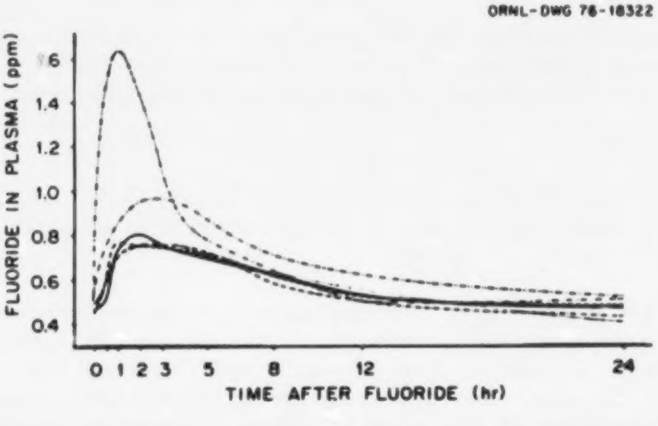

Figure 5.6. Diurnal variation in plasma fluoride in sheep. Each curve represents a single animal. Source: Adapted from Simon and Suttie, 1968, Figure 1, p. 512. Reprinted by permission of the publisher.

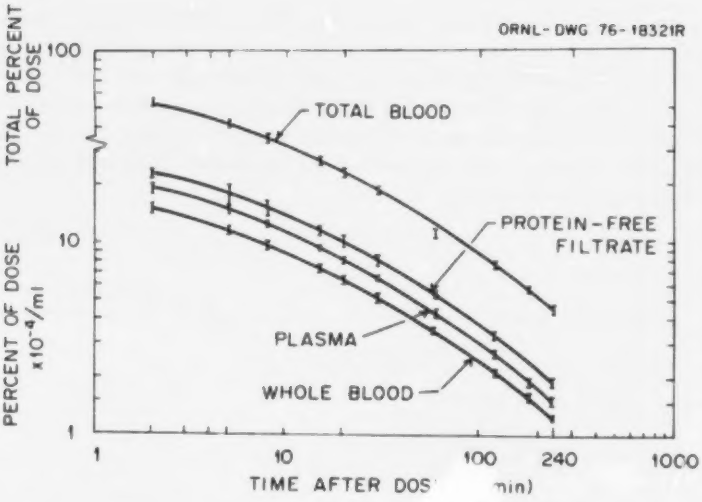

Figure 5.7. Fluorine-18 levels in blood from mature cows after intravenous dosing. Source: Adapted from Bell, Merriman, and Greenwood, 1961, Figure 1, p. 380. Reprinted by permission of the publisher.

accumulation decreases with increased fluoride bone content and age of animal.

Fluoride deposition in bone varies with the kind of bone and with the area of bone involved. Some bones tend to have greater fluoride-induced exostosis formation than others, such as the mandibles and shaft bones (Underwood, 1971). Active areas of bone growth have a high rate of fluoride incorporation, cancellous areas incorporate fluoride faster than cortical areas, and surface areas faster than middle regions. Kay (1975) reported that in mule deer the cancellous portions of bones and the bone sections with the largest surface-area-to-weight ratio had the highest fluoride concentrations.

Fluoride is incorporated into teeth as fluorapatite during calcification; once calcified, the tooth does not respond to fluoride as the bone does (National Academy of Sciences, 1974). Dentin fluoride concentrations normally increase with time.

Dairy cows fed a basal ration containing 3 to 5 ppm fluoride for 5.5 years had less than 1000 ppm fluoride (dry, fat-free weight) in the metacarpal, metatarsal, mandible, maxilla, and frontal bones (Suttie, Phillips, and Miller, 1958). When 20 and 50 ppm fluoride were added to the diet, fluoride bone content increased 4.5 to 10 times, respectively, over the basal diet controls.

The form of fluoride ingested influences the amount of fluoride deposited in the bones (Ammerman et al., 1964). Calves and steers fed for 91 days on different sources of fluoride had higher fluoride deposition from consuming soft phosphate (134 ppm fluoride) than those consuming half the equivalent level of supplemental fluorine as sodium fluoride (67 ppm). More fluoride was deposited from 67 ppm fluoride as sodium fluoride than from 134 ppm as calcium fluoride (Figure 5.8). Mule deer inhabiting areas with vegetation containing high fluoride levels had 5- to 50-fold increases in bone fluoride content as compared with deer from an uncontaminated area (Kay, Tourangeau, and Gordon, 1975*b*).

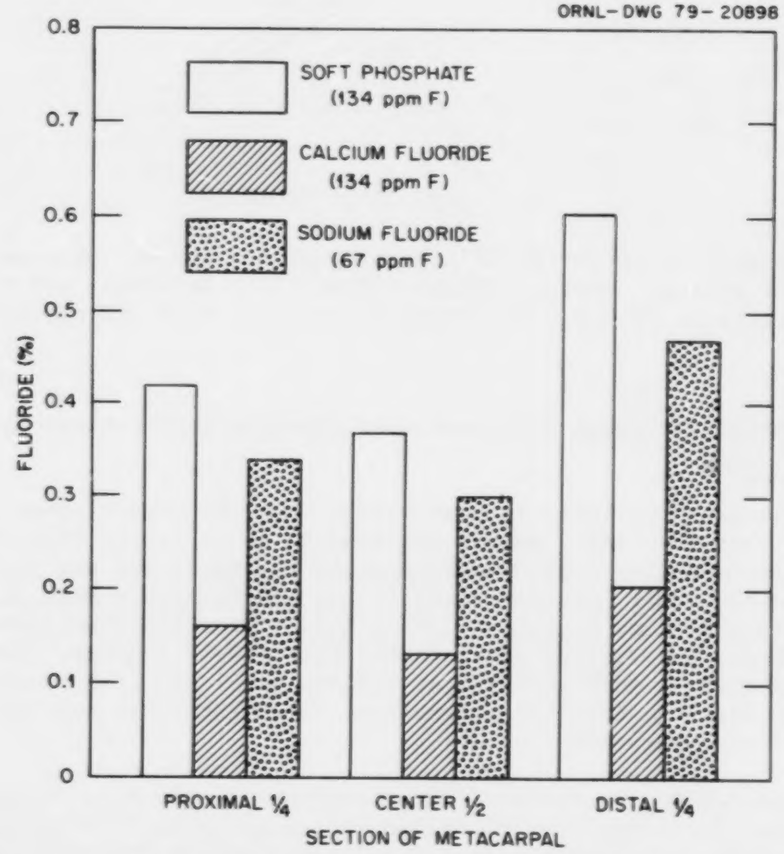

Figure 5.8. Effect of fluoride source on fluoride deposition (expressed as percentage of bone ash) in three sections of the metacarpal bones of cattle. Source: Adapted from Ammerman et al., 1964, Figure 1, p. 412. Reprinted by permission of the publisher.

Examination of incisor teeth of cattle in fluorosis and nonfluorosis areas showed that a relationship existed between dental changes and fluoride content of teeth (Mortenson et al., 1964). Fluoride levels were higher in the dentin than in the enamel; average fluoride content of enamel was 37% to 43% of that in dentin. The enamel acquired its fluoride before the incisors erupted from the gums. The dentin continued metabolic activity and accumulated fluoride much like bones. As the fluoride concentration and tooth injury increased, the amount of enamel in the tooth decreased.

5.5.1.2.3.3 Soft tissues — Fluoride concentrations in soft tissues are low and do not increase significantly with age (Underwood, 1971). Soft tissues begin to increase in fluoride content when the bone reaches a fluoride saturation point (Lillie, 1970). Kidneys have the highest fluoride concentration of soft tissues because of urine retained in the tubules and collecting ducts. The placenta, aorta, and tendon have higher fluoride levels than most other soft tissues, probably as a result of pathologic calcification, which binds fluoride to the tissue (National Academy of Sciences, 1974).

The fluoride concentration in soft tissues of control cows fed a basal ration containing 3 to 5 ppm fluoride for 5.5 years was 2 to 3 ppm (Suttie, Phillips, and Miller, 1958). When 50 ppm dietary fluoride was added, tissue fluoride content increased (Table 5.21). The increase in tissue fluoride concentration was not significant enough to use soft-tissue analyses as a criterion of fluorosis. Bell, Merriman, and Greenwood (1961) reported that stable fluoride concentrations in soft tissues varied with the level of fluoride ingested by cows; however, the increase in tissue-fluoride levels with increased fluoride intake was small.

5.5.1.2.4 Placental transfer — Some transfer of fluoride across the placenta appears to take place, although there may be species variation in the amount (National Academy of Sciences, 1974). Shupe, Harris, Greenwood, Butcher, and Nielsen (1963) found that in the fetus, fluoride was deposited in teeth and bones. Newell and Schmidt (1958) showed a selective deposit of fluoride in the metacarpal bones of newborn calves from cows fed increased levels of fluoride. As the fluoride intake of the cow increased, the fluoride concentration of the fetal metacarpal bones increased (Table 5.22), indicating transfer of fluoride from cow to fetus. Greenwood et al. (1964) reported a correlation between fluoride bone content of calves and the amount of fluoride ingested by the dam. However, calf bone-fluoride content did not increase in succeeding gestations.

The distribution of intravenously administered ^{18}F to pregnant cows indicated that part of the ^{18}F passed into the fetus, but there was a barrier for the free passage of fluoride (Bell, Merriman, and Greenwood, 1961). The maternal placenta contained 0.60% of the dose, compared with 0.25% in the fetal placenta.

Bawden, Wolkoff, and Flowers (1964) injected sheep with ^{18}F to study maternal-fetal fluoride metabolism. Maternal plasma clearance of ^{18}F was rapid, and fetal plasma concentrations were low when compared with maternal-plasma levels. This suggested that the placenta may limit the transfer of fluoride to the fetus. Fluorine-18 injected into the fetus of a pregnant sheep was found in the maternal plasma; this result demonstrated that fluoride can be transferred from the fetus to the mother.

TABLE 5.21. THE EFFECT OF ADDED DIETARY INCREMENTS OF FLUORIDE (AS SODIUM FLUORIDE) ON SOFT TISSUE FLUORIDE CONCENTRATIONS IN DAIRY COWS

Lot	Fluoride added (ppm)	Cow No.	Fluoride concentration (ppm)					
			Heart[a]	Liver[a]	Kidney[a]	Pancreas[b]	Thyroid[b]	Adrenal[b]
I	0	2	1.8	3.1	3.1	1.7	0.6	2.0
		3	3.3	1.9	2.9	4.0	2.7	11.9
		4	1.7	1.9	4.4	2.0	2.9	2.2
		Av	2.3	2.3	3.5	2.8	2.1	5.3
II	20	5	2.7	2.4	7.3	1.4	2.9	3.8
		6	4.4	2.1	6.0	2.6	7.0	
		7	2.5	2.3	8.3	3.2	6.6	
		8	4.0	3.9	12.8	1.7	11.2	3.3
		Av	3.4	2.7	8.6	2.2	6.9	3.5
III	30	10	2.7	2.5	12.5	2.0	2.4	3.3
		11	4.7	4.6	9.1	5.0	4.1	
		12	3.0	5.1	10.5	3.5	4.1	3.3
		Av	3.5	4.1	10.7	3.5	3.5	3.3
IV	40	13	3.3	5.6	19.7	3.0	4.9	2.5
		14	5.5	3.3	20.4	5.1	5.0	3.5
		15	4.5	3.4	7.8			
		16	2.9	3.0	16.0	3.4	7.6	8.8
		Av	4.0	3.8	16.0	3.8	5.8	4.7
V	50	17	5.0	2.3	13.7	4.5	5.2	8.7
		19	3.2	2.1	15.4	4.1	12.2	
		20	6.3	4.8	28.9	4.0	4.4	4.1
		Av	4.6	3.6	19.3	4.2	7.3	6.4
VI	50 + $CaCO_3$	22	3.8	3.5	11.1	3.6	4.2	
		23	4.6	2.7	7.7	3.5	4.9	4.2
		24	5.6	2.8	8.4	3.0	9.0	4.1
		Av	4.6	3.0	9.0	3.4	6.0	4.1

[a] Dry weight.
[b] Dry, fat-free weight.

Source: Adapted from Suttie, Phillips, and Miller, 1958, Table 2, p. 299. Reprinted by permission of the publisher.

TABLE 5.22. FLUORIDE CONCENTRATION IN METACARPAL BONES OF NEWBORN CALVES

Total daily fluoride fed cows (ppm body wt)	Average fluoride in fetal metacarpal bones[a] (ppm)
0.15-0.3	43
1.0	99
1.5	113
2.0	152
2.5	165

[a] Dry, fat-free basis.

Source: Adapted from Newell and Schmidt, 1958, Table 8, p. 369. Reprinted by permission of the publisher.

5.5.1.3 Elimination — Fluoride is excreted in urine, feces, and sweat. Traces of fluoride are also found in hair, saliva, and milk. The major route of excretion, urine, is influenced by total fluoride intake, form of fluoride taken in, frequency and duration of exposure, fluoride content of the bone, general health, and other factors. Except during excessive sweating, only very small amounts of fluoride are eliminated in perspiration.

In the urine and feces, the relative proportions of excreted fluoride are dependent on the type of fluoride compound ingested, its solubility, the initial amount ingested, and the method of ingestion (Underwood, 1971). Soluble fluorides are excreted in the urine. Smaller amounts of the less soluble fluorides, such as calcium fluoride, appear in urine as a result of lower absorption. Sheep and cows usually excrete 50% to 90% of dietary fluoride in the urine. Bell, Merriman, and Greenwood (1961) reported that most of the ^{18}F added to the basal ration of beef cattle was excreted in the urine (Figure 5.9). The average urinary ^{18}F level reached a peak of 40.4×10^{-4} percent of dose per milliliter 15 min after dosing. Saliva peaked at 1.3×10^{-4} percent per milliliter after 5 min, and ^{18}F in feces was 4.1×10^{-4} percent per milliliter at 180 min.

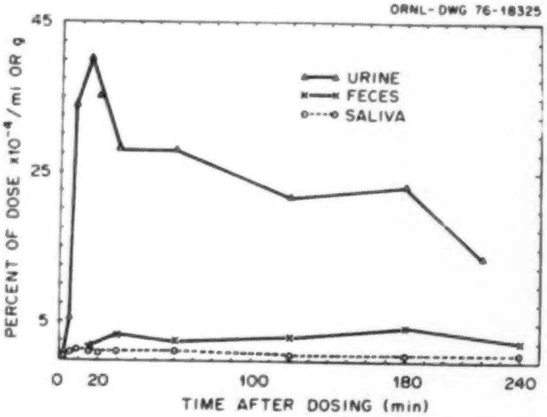

Figure 5.9. Average fluoride-18 concentration in excreta and saliva from mature cows. Source: Adapted from Bell, Merriman, and Greenwood, 1961, Figure 2, p. 381. Reprinted by permission of the publisher.

Urinary fluoride levels correlate positively with the levels of ingested fluoride, the amount of fluoride stored in skeletal tissue, and the age of the animals. Sheep and cattle not exposed to excess fluoride had urinary fluoride concentrations close to 5 ppm (Underwood, 1971), but with elevated fluoride intake, urinary fluoride levels increased to 15 to 30 ppm and in some cases reached as high as 70 to 80 ppm. Shupe, Harris, Greenwood, Butcher, and Nielsen (1963) reported an increase in urine fluoride content with increased fluoride in consumed dry matter (Figure 5.10).

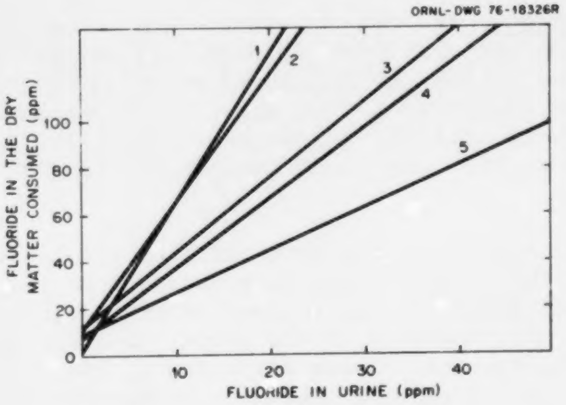

Figure 5.10. Relationship of urinary fluoride level to the fluoride in dry matter consumed by cows. Source: Adapted from Shupe, Harris, Greenwood, Butcher, and Nielson, 1963, Figure 1, p. 301. Reprinted by permission of the publisher.

Also, as the animals increased in age, more fluoride was excreted in the urine in proportion to the fluoride in the dry matter. The increase of urinary fluoride levels with age results from less storage of ingested fluoride in the bones of older animals as the bone reaches a saturation level (National Academy of Sciences, 1974). Portions of fluoride stored in the skeleton can later be mobilized and excreted after the animal has been removed from a high-fluoride diet (Phillips, Suttie, and Zebrowski, 1963). Cattle given a normal dairy ration for two years following four years on high-fluoride diets excreted portions of their skeletal fluoride load. Therefore, urinary fluoride may arise from the release of skeletal fluoride as well as from fluoride being ingested in feed and water.

5.5.2 Effects

Animals can usually ingest small amounts of fluoride without any harmful effects; however, the intake of excessive quantities induces various deleterious effects. Damage to animals in the field often results from ingestion of high-fluoride—containing feed contaminated by industrial pollution or by the addition of high-fluoride—containing phosphates to animal diets. Fluorides in excessive amounts can inhibit several enzyme systems and impair appetite, resulting in reduced growth and milk production.

Both acute and chronic effects can result from fluoride exposure. Acute toxicity results in muscular weakness, convulsions, and cardiac and respiratory failure. Chronic toxicosis is caused by ingestion of sublethal fluoride amounts over long periods of time. The terms fluorosis, fluorine toxicity, fluoride toxicity, fluorine toxicosis, and fluoride intoxication have been used interchangeably by some individuals, with no distinction made among them.

5.5.2.1 Physiological Effects — Exposure to excessive fluoride levels inhibits specific enzymes involved in vital processes (National Academy of Sciences, 1971). Fluoride inhibits cholinesterase, which may be partially responsible for effects on the nervous system. Fatty acid oxidase activity is also inhibited, causing a blockage of fatty acid utilization (Underwood, 1971). Carbohydrate metabolism is altered and the level of glucose-6-phosphate dehydrogenase decreased in some fluorotic animals.

Bone alkaline phosphatase activity is related to fluoride intake and degree of fluoride-induced bone changes. In cattle, Greenwood et al. (1964) reported a correlation among bone alkaline phosphatase activity, level of fluoride intake, fluoride content of bone, and degree of osteofluorosis. Phosphatase activity increased with increasing amounts of ingested fluoride. There were no effects on lipase and amylase in the pancreas, succinic dehydrogenase in heart muscle, enolase and pyruvate kinase in the skeletal muscle, and pepsin in abomasal mucosa.

Faccini and Care (1965) reported changes in the ultrastructure of parathyroid glands in sheep given 200 ppm sodium fluoride in drinking water for one week and four weeks. An immunoassay showed that there was a fivefold increase in the amount of blood parathyroid hormone after one week of ingesting the fluoride. These results indicate that the parathyroid glands are hyperactive in severe skeletal fluorosis.

5.5.2.2 Acute Toxicity — Acute fluoride toxicosis is relatively rare and has not been intensely investigated. Acute fluoride toxicosis usually is the result of accidental ingestion of compounds such as sodium fluorosilicate, sodium fluoride (National Academy of Sciences, 1974), sodium fluoroacetate ("1080"), and fluoroacetamide (Shupe, 1969).

Various types of toxic responses occur, depending on several factors. The rapidity of symptom appearance is dependent on the amount of fluoride ingested. Symptoms may appear within 30 min for excessively high amounts (Shupe, 1969). Symptoms usually present are: high fluoride levels in blood and urine, stiffness, restlessness, anorexia, reduced milk production, nausea and vomiting, excessive salivation, chronic convulsions, necrosis of mucosa of digestive tract, weakness, depression, and cardiac failure (National Academy of Sciences, 1974). In 1927, Krug (as cited in Shupe and Alther, 1966) reported cows dying 18 to 24 hr after ingestion of sodium fluorosilicate added mistakenly to the diet. Convulsions, exaggerated chewing motions, and hyperemia were observed.

Cass (1961) reviewed cases of acute fluorosis in livestock. Responses to various levels of fluorides inducing acute effects are given in Tables 5.23-5.25.

Egyed and Shlosberg (1975) reported 27 field cases of acute sodium fluorosilicate poisoning in domestic animals. The affected cattle displayed drowsiness, loss of appetite, constipation, paresis of the rumen, and severe abdominal pain. Sheep showed grinding of the teeth and frothing at the mouth, and bradycardia occurred in horses. Under experimental conditions, mild symptoms of acute sodium fluorosilicate poisoning appeared

TABLE 5.23. RESPONSE OF DOMESTIC ANIMALS TO VARIOUS LEVELS OF FLUORIDE DOSAGE: MINIMAL RESPONSE

Compound	Species of animal	Fluoride concentration (ppm)	Daily fluoride dose	Schedule
			Acute signs not induced	
NaF	Cattle	300 and less	∿4.12 mg/kg and less	In diet, several months
	Swine	∿7.0–90.5		In feed, ad lib 75 days
	Sheep		1.83 and 2.35 mg/kg	In capsule orally, 1 time
	Horse		13.8–41.4 mg/kg	
	Horse		11 mg/kg	In capsule, every other day, 3 doses
Na_2SiF_6	Cattle	∿196–982	196–982 mg per animal	In molasses and bran, 1 time
	Sheep	24,249	12 g per animal	In bran (refused feed) force fed, 1 time
Ca phosphate (1–3% NaF)	Horse		1.14–1.9 g per animal or 2.54–4.22 mg/kg	In the diet for 11 days
$3NaF \cdot AlF_3$ (synthetic cryolite)	Cattle	10,860 and 27,150	25.3, 38.8, and 51.2 mg/kg	Daily in water, orally 28, 30, and 15 days respectively
	Sheep		599 mg/kg	In capsule, 1 time (1 sheep)
AlF_3	Swine	20,361	469 and 1730 mg/kg	In feed, 1 day (2 pigs)
$Al_2(SiF_6)_3$		14,246	1210 mg/kg	In feed, 1 day (1 pig)
BaF_2		4,334	178 mg/kg	In feed, 1 day (1 pig)
$3NaF \cdot AlF_3$		27,145	919 and 1196 mg/kg	In feed, 1 day (3 pigs)
			Marginally acute signs induced	
NaF	Cattle	302–<453	0.9–1.36 g per animal	Daily in water, stomach tube, several days
		2,262 and 6,786	5.27 and 12.8 mg/kg	In water, orally, once daily 28 and 15 days respectively
			10.3 mg/kg	In feed, daily during 12 days omitting days 6 and 11
	Horse		19.9 mg/kg	In capsule, daily for 2 days
			7.9 mg/kg	In feed, daily for 12 days
Na_2SiF_6	Cattle	403–<909	1.2 to <1.82 g per animal	In water by stomach tube for 2 to 3 months

Source: Adapted from Cass, 1961, Table I, p. 474. Data collected from several sources. Reprinted by permission of the publisher.

in 1- to 3-year-old female sheep given oral doses of 25 to 50 mg/kg body weight. With a 200-mg/kg dose severe symptoms occurred, followed by death on the sixth day after poisoning. Death occurred 3.0 and 2.5 hr after administration of 1.5 and 2.0 g of fluorosilicate per kilogram respectively. In fatal poisoning, hypocalcemia; hypomagnesemia; increased blood sugar; and increased blood phosphorus, glutamate oxaloacetate transaminase, isocitric dehydrogenase, and lactate dehydrogenase were found. These findings indicated an effect on calcium and magnesium metabolism and cellular malfunction.

5.5.2.3 Chronic Toxicity — Chronic fluorosis results from long-term excessive fluoride ingestion in livestock and wildlife. Industrialization (e.g., factories that manufacture aluminum, steel, or phosphate or factories that heat ores to high temperatures) has made fluoride toxicosis in livestock an important problem in the United States as well as other countries (Gründer, 1972; Milhaud and Godfrain, 1975; Shupe, 1970; Suttie, 1969). The onset of fluoride toxicosis is usually insidious, and symptoms may be confused with other element toxicoses or deficiencies. Fluoride toxicosis is influenced by the amount of fluoride ingested, duration of

TABLE 5.24. RESPONSE OF DOMESTIC ANIMALS TO VARIOUS LEVELS OF FLUORIDE DOSAGE: ACUTE RESPONSE

Compound	Species of animal	Fluoride concentration (ppm)	Daily fluoride dose	Schedule
NaF	Cattle	600 and more	~6.13 mg/kg or more	In feed for 2 to 3 days
		453-903	1.36-2.71 g per animal	Daily in water, stomach tube, a few days
			37.5 mg/kg	In grain feed, 1 time
		13,572	23.6 mg/kg	In water, orally, once daily for 27 days
	Swine	4,524 and more		In feed for 1 to 3 days
		18,096	153 mg/kg	In feed, for 1 meal
		>90.5		Reduced intake of feed, 75 days
	Sheep		136 mg per animal	In mixture of milk and water, orally, 1 time
			136 mg per animal	In mixture of milk and water, orally, 1 time
	Goat	4,524	11.9 and 12.6 mg/kg	In water, stomach tube, daily 6 days
			73.7 mg/kg	In capsule, 1 time
			166 mg/kg	In grain feed, 1 time (refused feed)
	Horse		46.0 and 55.2 mg/kg	In feed for 1 day
			22.6 g per animal	In grain feed, 1 time (refused feed)
Na_2SiF_6	Cattle	909-1,212	1.82-3.64 g per animal	In feed for a few days
			1.22-2.43 g per animal	In distilled water for a few days
	Swine		121-668 mg/kg	In feed for 1 meal
	Goat	606		In feed for 4 days
			69 mg/kg	In feed for a few days
Ca phosphate (2-3% NaF)	Swine		190-304 mg per animal	In feed for 22 days
		225.8 and 248.8		In feed for a few days
	Horse		3.4 g per animal increased to 3.8 g	Lower dose (7.57 mg/kg) increased after 2 days to higher dose (8.44 mg/kg) for 2 days
$CaSiF_6$	Cattle	1,250 and 2,500	1.25 and 2.5 g per animal (intended)	In feed (most of feed refused)
$3NaF \cdot AlF_3$ (synthetic cryolite)	Cattle	43,400 and 54,300	46.4, 63.9, and 94.4 mg/kg	In water, orally, once daily, 16, 72, and 27 days respectively
BaF_2	Swine	6,501	267 mg/kg	In feed for 1 day (1 pig)

Source: Adapted from Cass, 1961, Table II, p. 474. Data collected from several sources. Reprinted by permission of the publisher.

ingestion period, fluctuations of fluoride-ingestion level with time, fluoride solubility, species of animal, age during fluoride ingestion, level of nutrition, stress factors, and individual response (National Academy of Sciences, 1974). Chronic fluorosis may be difficult to diagnose, and evaluation should be based on clinical, histopathological, radiographical, and necropsy findings (including chemical analyses). In particular, the following combination of factors is of prime importance in diagnosing chronic effects: (1) the extent of dental fluorosis, (2) the extent of osteofluorosis, (3) the levels of fluoride in the soft tissues, body fluids, mineralized tissues, and dietary rations, and (4) the occurrence and degree of intermittent lameness (Shupe, Olson, and Sharma, 1979).

5.5.2.3.1 Dental fluorosis — One of the most sensitive reactions to fluoride toxicosis occurs in teeth (Shupe and Alther, 1966). When evaluated fluoride levels are ingested during tooth formation, both enamel and dentin

TABLE 5.25. RESPONSE OF DOMESTIC ANIMALS TO VARIOUS LEVELS OF FLUORIDE DOSAGE: LETHAL DOSES

Compound	Species of animal	Fluoride concentration (ppm)	Daily fluoride dose	Schedule
NaF	Cattle (calf)		45.2 mg/kg	Orally, 1 time
	Swine	22,620	293 and 554 mg/kg	In feed for 1 day
	Sheep		86.6 and 129.8 mg/kg	In capsule, 1 time
			15 mg/kg	In feed for 51 days
	Goat		99.5-199 mg/kg	In capsule, 1 time
			285 mg/kg	In feed for 1 day
	Horse		>45 g/kg	Orally, 1 time
Na_2SiF_6	Sheep	24,248	24.1 g per animal	In bran (refused feed, so force fed), 1 time
			334 mg/kg	In capsule, 1 time
	Goat		1.82 g per animal	In feed for 1 meal
			82-90 mg/kg	In feed for a few days (calculated)
Ca phosphate (2-3% NaF)	Swine		304 mg increased to 380 mg per animal	In feed (low dose 24.4 mg/kg for 4 days increased to 30.4 mg/kg for 5 days)
$3NaF \cdot AlF_6$ (synthetic cryolite)	Sheep		1 g/kg	Orally, 1 time

Source: Adapted from Cass, 1961, Table III, p. 475. Data collected from several sources. Reprinted by permission of the publisher.

are adversely affected. Schmidt, Newell, and Rand (1954) found that the maximum effect of fluoride on incisor enamel of cattle fed 1.0 to 2.5 mg/kg fluoride daily, occurred approximately 9 to 12 months prior to tooth eruption. Affected teeth show characteristic fluorotic lesions such as mottling, staining, hypoplasia, hypocalcification, and excessive abrasion (Shupe, 1969). Specific damage varies in degree as influenced by the fluoride level intake. For the purpose of classification, the following standards are used in determining the severity of dental fluorosis: (0) normal — smooth, translucent, glossy white appearance, normal shape; (1) questionable effect — slight change from normal, cause may or may not be determined, enamel flecks but no mottling; (2) slight effect — slight mottling as horizontal striations, normal wear rate; (3) moderate effect — definite mottling and staining, large areas of chalky enamel, slightly increased wear rate; (4) marked effect — definite mottling, staining, hypoplasia, hypocalcification, enamel may be pitted, increased wear rate; and (5) severe effect — definite mottling hypoplasia, hypocalcification, excessive wear rate, and erosion or pitting of enamel.

Histological changes in the dental fluorosis are: the ameloblasts are prematurely reduced in size and filled with basophilic-staining globules; epithelial papillae are abnormal; enamel epithelium has series of interruptions in its smooth surface; and the abnormal enamel matrix does not calcify normally (National Academy of Sciences, 1971). Affected teeth are subject to more rapid wear and erosion of enamel away from the dentin. The pattern of excessive or accelerated teeth erosion is important in determining the relative loss in effective use and length of service of the teeth (Garlick, 1955). A level of 2 ppm fluoride in livestock drinking water may cause tooth mottling; however, this is the upper limit of fluoride in drinking water recommended for livestock (National Academy of Sciences and National Academy of Engineers, 1972).

Dental fluorosis caused by ingestion of mineral supplements containing high fluoride levels has been reported. Cattle ingesting a daily total of 5976 ppm fluoride from mineral supplements during tooth development showed badly affected incisors (Dale and Crampton, 1955). Neeley and Harbaugh (1954) detected distinct evidence of dental effects (mottling, staining, and abnormal wear) in cattle exposed to fluoride in their diet for six years at levels of 0.52 to 1.69 mg/kg body weight. Adding 20 to 50 ppm fluorine as sodium fluoride to the basal ration of cattle caused slight to severe mottling and wear of teeth respectively (Suttie, Miller, and Phillips, 1957). The addition of 200 g of calcium carbonate had a slight alleviating effect. Suttie and Faltin (1971) later exposed dairy heifers (by ingestion) to 2.5 mg of fluoride per kilogram body weight during their 13th to 15th or 16th to 18th month of age. Dental fluorosis in at least one pair of incisors was evident, especially in the heifers exposed during their 16th to 18th month of age. The most severe dental effects were observed when exposure coincided with the initiation of crown formation and calcification for a particular incisor.

Greenwood et al. (1964) reported that dental effects or lesions were indicative of exposure of cattle to fluorine during the time of tooth formation and calcification. They found that positive correlations existed between dental lesions of certain incisor teeth and degree of abrasion of certain molar teeth. The first incisor correlated with the second molar, the second incisor with the third molar, and the third incisor with the second premolar.

Shupe, Miner, Greenwood, Harris, and Stoddard (1963) found that cattle fed a diet containing 49 ppm fluoride had moderate to marked dental effects, whereas a diet containing 93 ppm fluoride resulted in severe dental fluorosis. Calcium fluoride fed at a level to constitute 69 ppm of the total diet plus low-fluoride—containing hay (10 ppm fluoride) caused less severe effects on incisor teeth of dairy heifers than did sodium fluoride fed at the same level plus low-fluoride—containing hay (Shupe et al., 1962). This result indicated that calcium fluoride is less toxic than sodium fluoride. Horses grazing in areas where cattle and sheep had developed severe fluorosis showed characteristic dental lesions. However, the horses had to ingest larger amounts of fluoride than cattle or sheep before these symptoms became evident (Shupe and Olson, 1971).

Chronic fluorosis was found in a herd of sheep in the Vredenburg district, Cape Province, South Africa (Zumpt, 1975). The sheep had protruding hipbones, sharp vertebral columns, and poor muscular development. Incisors were pitted and showed irregular severe wear. Pasture samples contained 232 ppm fluoride, the source of which was rock phosphate dust from a fertilizer factory.

In cattle grazing in fluorotic and nonfluorotic areas, a close relationship was found among lesions of incisors, fluorine content of teeth, and amount of enamel (Mortenson et al., 1964). As the effect of incisors increased in severity, fluoride levels increased and the percentage of enamel decreased. The enamel acquired its fluoride before the incisors erupted from the gums. Table 5.26 illustrates the dental effects of various fluoride concentrations on several species of livestock.

Examining cattle from areas receiving various fluoride levels from industrial sources, Wohlers and Newell (1964) found a correlation between severity of teeth effects and distance from the fluoride source. Almost 50% of incisor pairs within a distance of 2 miles from the source were classified 4 and 5, whereas at a distance greater than 2 miles, only 5% were classified 4 and 5 (Figure 5.11).

5.5.2.3.2 Osteofluorosis — Prolonged ingestion of low fluoride levels does not produce adverse effects on bone structure (Shupe and Alther, 1966); however, the intake of excessive levels of fluoride for long time periods will induce characteristic lesions (National Academy of Sciences, 1974). Bones with fluoride-induced periosteal hyperostosis are chalky white, rough, and porous. Mineralization of the tendons at the point of attachment to the leg bones can occur. The severity of the lesions is partially related to the stress and strain imposed on the bones (National Academy of Sciences, 1974).

Three mechanisms are involved in the production of osteofluorosis; (1) impairment of the mechanical properties of normal bone, (2) decompensation of bone function by remodeling, and (3) direct inhibition of normal osteoblastic activity by excessive fluoride levels (Hodge and Smith, 1965). Table 5.27 describes the bone lesions associated with exposure of livestock to various levels of fluoride.

Shupe, Miner, Greenwood, Harris, and Stoddard (1963) reported marked bone changes in cows fed 49 to 93 ppm fluoride. Lesions occurred after 1.5 to 2 years in cows fed 93 ppm and 3.5 to 4 years in cows fed 49 ppm. The first lesions appeared on the medial surface of the proximal third of the metatarsal bones and were bilateral. The authors suggested that abnormal osteoblastic activity results in formation of abnormally poor organic matrix with faulty and irregular calcification. Table 5.28 presents data showing the relationship between fluoride concentration in the diet and severity of osteofluorosis and ankylosis of the metacarpus and metatarsus bones. Jones (1972) reported lesions of the bones and arthritis in the hips of cows, attributing these effects to mineral supplements high in fluorine. Analysis of feed showed that the intake of fluorine was 50 to 70 ppm of the total dry matter. However, other researchers have not incriminated elevated fluoride intake with uncomplicated degenerative osteoarthritis (Shupe, Olson, and Sharma, 1972). Fluoride-induced bone lesions do not initially or primarily affect articular surfaces, but secondary changes and overgrowth may affect joint movement in severe cases.

Bélanger et al. (1958) found defective growth and mineralization of bones, costochondral beading, softened and deformed epiphyseal plates,

TABLE 5.26. DENTAL LESIONS ASSOCIATED WITH EXPOSURE OF LIVESTOCK TO ELEVATED FLUORIDE LEVELS

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure	Age at start of exposure	Dental lesions
Cattle	Soluble fluoride in feed	≳15 to 25	Av 0.4-0.6 mg/kg		Nil to slightly moderate markings and stain; no abnormal wear
	Natural fluoride in drinking water	5	Av ≳0.95 mg/kg		Changes comparable with those associated with 15 to 25 ppm in feed, above
		144			Changes comparable with those associated with 15 to 25 ppm in feed, above; 60% of 35 cattle showed these lesions
	Soluble fluoride in feed	40	Av ≳0.88-1.26 mg/kg		Medium to well marked; no abnormal wear
		50	Av ≳1.2-1.64 mg/kg (peak 1.91)		More marked than at 40 ppm; no particularly abnormal wear
	Natural fluoride in drinking water	16			Changes comparable with 50 ppm in feed above
	Soluble fluoride in feed	Estd 77 or more	Av ≳1.7-2.5 mg/kg		Severe dental changes marked mottling, staining and abnormal wear on some animals
	Natural fluoride in drinking water	Estd 18 or more	Av ≳1.7-2.5 mg/kg		Severe dental changes marked mottling, staining and abnormal wear on some animals
	Raw rock phosphate in feed		1.2 and 2.0 mg/kg		Slight to moderate
		≥200	≳3.5-4 mg/kg		Severely marked
	Pasturage contaminated with fluoride-containing atmospheric waste from industry	12-14			Nil
		27-44			Slight to moderate
		40-60			Marked to severely marked
	Pasturage contaminated with fluoride-containing atmospheric waste from phosphate-producing industry		5 mg/kg		Severely marked (exposure included tooth-forming period)
			5 mg/kg		Nil (exposed after tooth-forming period)
	Na_3AlF_6 in feed		60 mg per animal per day for 195 days	1 calf	Moderate
Sheep	NaF in feed		15 mg per animal per day for 71 days	1 lamb	Moderate

TABLE 5.26 (continued)

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure	Age at start of exposure	Dental lesions
	CaF₂ in feed		340 mg per animal		Well marked
	Raw rock phosphate in feed		7 mg per animal for 3 years	After 1 year old	Nil
			60 mg per animal for 3 years	After 1 year old	Mild to moderate
			120 mg or more per animal for 3 years	After 1 year old	Severe
			1.5	Feeder yearlings	Slight discoloration
			6.0		Fracturing and erosion
	NaF added to drinking water	5	Winter: av 9 mg per animal, $\simeq$0.29 mg/kg; summer: av 13 mg per animal, $\simeq$0.37 mg/kg for 3.5 years	After 1 year old	Nil to very slight mottling; no abrasion (molar teeth fluoride content = 1300 ppm)
		≤7		3 to 6 months for 2 years	Nil
		10	Winter: av 18 mg per animal, $\simeq$0.57 mg/kg; summer: av 27 mg per animal, $\simeq$0.71 mg/kg for 3.5 years	At 13 to 14 months (animals penned)	Moderately severe, selective abrasion (molar teeth fluoride content = 1900 ppm)
		20	Winter: av 37 mg per animal, $\simeq$110 mg/kg; summer: av 53 mg per animal, $\simeq$1.45 mg/kg for 3.5 years	At 13 to 14 months (animals penned)	Severe (molar teeth fluoride content = 5100 ppm)
		0.3		From birth, on pasture almost 7 years	Nil (molar teeth fluoride content = 300 ppm)
		10.0		From birth, on pasture almost 7 years	Slight mottling and selective abrasion (molar teeth fluoride content = 2100 ppm)
		20.0		From birth, on pasture almost 7 years	Severe (molar teeth fluoride content = 2700 ppm)
		10.0		At 2.5 to 3.5 years of age on pasture for 2 years	Nil (molar teeth fluoride content = <1000 ppm)

TABLE 5.26 (continued)

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure	Age at start of exposure	Dental lesions
		20.0		At 2.5 to 3.5 years of age on pasture for 2 years	Nil (molar teeth fluoride content = <1000 ppm)
		10.0		At 3 months; alternating 3 months exposed, 3 not	Slightly moderate, some weakening of cutting surfaces
		10.0		6 months exposed, 6 not	Moderately severe, some fractures
		10.0		6 months exposed, 3 not	Moderate, some wear
	Natural fluoride in groundwater for drinking purposes	$\simeq$6.0		From birth irregularly for 1 year	Chipping and mottling
		$\simeq$12.0			Severe, marked wear on central incisors
		12.0 (at time of survey)	Calcd: 43 to 73 mg per animal	From birth for 6 years	20% of flock with badly worn and broken teeth
	Pasturage contaminated with fluoride-containing atmospheric waste from industry	$\simeq$61.0		From birth	100% of flock severely affected
		$\simeq$44.0		From birth	∼60% severely affected
		$\simeq$16.0		From birth	High percentage of flock with much less effect than at 44 ppm above
Swine	NaF added to feed	290		From weaning for 2 or more years as reproducing females	Moderate to severe
		580		From weaning for 2 or more years as reproducing females	Moderate to severe
	NaF or Na_2SiF_6 added to feed		15 mg per animal, for 117 days	8 weeks old	Moderate
	Raw rock phosphate added to feed	330		From weaning for 2 or more years as reproducing females	Moderate to severe

TABLE 5.26 (continued)

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure	Age at start of exposure	Dental lesions
		650		From weaning for 2 or more years as reproducing females	Moderate to severe
		569	$\simeq$19-26 mg/kg	Young pigs for 2 years	Severe
		140		Young pigs for 2 years	Moderate

Source: Adapted from Cass, 1961, Table V, pp. 530-531. Data collected from several sources. Reprinted by permission of the publisher.

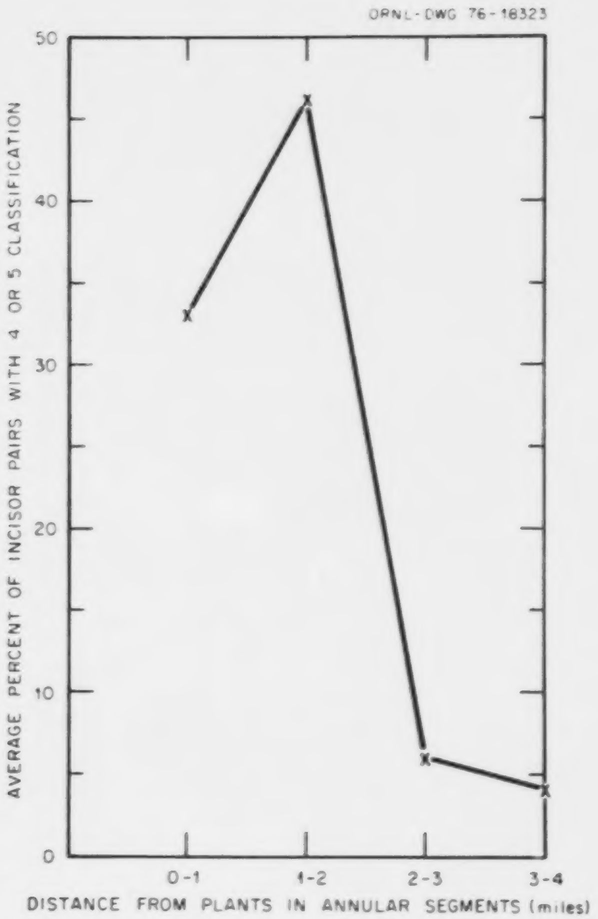

Figure 5.11. Variation of incisor classification of 4 or 5 with distance from industrial plants in annular segments. Source: Adapted from Wohlers and Newell, 1964, Figure 11, p. 148. Reprinted by permission of the publisher.

TABLE 5.27. SKELETAL LESIONS ASSOCIATED WITH EXPOSURE OF LIVESTOCK TO ELEVATED FLUORIDE LEVELS

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure	Age at start of exposure	Duration of exposure before appearance of lesion	Type of lesion
Cattle	NaF added to the feed	30	Av 0.63-0.91 mg/kg	2 years old for 5 lactations, dairy	5.5 years	Thin mantle of periosteal overgrowth on bones carefully cleaned at slaughter
		30	Av 0.63-0.91 mg/kg		3 years (no pasture)	Thin mantle of periosteal overgrowth on bones carefully cleaned at slaughter
		40	Av 0.88-1.26 mg/kg	4 to 6 years old (2 to 4 lactations) for an additional 3 lactations, dairy	3 years (no pasture)	Increasing roughness and thickening, some mineralization of periarticular structures and ligamentous attachments on bones carefully cleaned at slaughter
		50	Av 1.20-1.60 mg/kg	4 to 6 years old (2 to 4 lactations) for an additional 3 lactations, dairy	3 years (no pasture)	Increasing roughness and thickening, some mineralization and periarticular structures and ligamentous attachments on bones carefully cleaned at slaughter
		20	Av 0.40-0.61 mg/kg		3 years (no pasture)	Nil
		14-94	0.32-2.12 mg/kg	Calves, 3 months old	372 days	Rib biopsies gross and microscopic, nil
			$\sim$2.0 mg/kg	2 years old, dairy	2 years	Nil
			$\sim$2.5 mg/kg	2 years old, dairy	2 years	Slight osteofluorosis
		7		20 to 24 months old, beef	422 days	Nil
			$\sim$3.0-4.0 mg/kg	3 to 5 years old, hill cattle	Within 8 to 10 months	Osteofluorosis
		107	$\gtrsim$2.2 mg/kg	20 to 24 months old, beef	422 days	Osteofluorosis
	NaF in a capsule, orally	$\gtrsim$907 in feed	$\gtrsim$6.25 mg/kg	20 to 24 months old, beef	71 and 104 days respectively	Osteofluorosis
		$\gtrsim$1207 in feed	$\gtrsim$9.0 mg/kg	20 to 24 months old, beef	40 and 81 days respectively	Osteofluorosis
	Pasturage contaminated with fluoride-containing atmospheric waste from industry	Exposure uncalculated but elevated; hay: 20-80; dust on hay: 260-480		Various ages	After 2 to 3 months	Palpable osteofluorosis on ribs
	Rock phosphate in feed	$\geq$200	400 ppm estd as 6 mg/kg	Experimentally at 6 months old, clinically at various ages	$\sim$5 to 6 years	Osteofluorosis

TABLE 5.27 (continued)

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure	Age at start of exposure	Duration of exposure before appearance of lesion	Type of lesion
			Calcd as 1.2-2.0 mg/kg	As calves, and as lactating cows	Up to 5 3/4 years	Nil
	Natural fluoride in drinking water	25-32			Most of lifetime	Osteofluorosis
		14.4-18.6			Most of lifetime	Nil
	NaF added to drinking water		~4.91 and 5.35 mg per animal	2 calves, starting with 50 and 86 kg	10 months	Osteofluorosis
Sheep	NaF in feed		15.0 mg/kg	One 2.5 years old, one 6 months old	1 month	Osteofluorosis
		206		1 year old	140 days	Nil
		111	~30 mg/kg	1 year old	3 years	Nil
		112	~3.9 mg/kg	Weanlings	98 days	Nil
	Raw rock phosphate in feed		120 mg or less per animal	1 year old	3 years	Nil
			160-170 mg per animal	1 year old	3 years	Osteofluorosis
			Up to 6.0 mg/kg	Growing lambs	136 to 235 days	No osteofluorosis mentioned
			Up to 6.0 mg/kg	Ewes	2.5 to 3 years	No osteofluorosis mentioned
	NaF added to drinking water	10 and 20		13-14 months old, fed in pens	3.5 years	No osteofluorosis mentioned
		10 and 20		2.5-3.5 years old, grazing on pasture	2.6 months	No osteofluorosis mentioned
		10 and 20		13-14 months old, grazing on pasture	6 years	No osteofluorosis mentioned
Swine	NaF in feed	≥290		Weanlings	Within 144 days	Osteofluorosis
	Raw rock phosphate in feed	≥330		Weanlings	Within 144 days	Osteofluorosis
		≥293	≳11 to 14 mg/kg or more	Young pigs	5 years	Size, smoothness, texture and color of bones changes; no osteofluorosis mentioned
		140		Young pigs	5 years	No gross changes of bones
	NaF, Na_2SiF_6, Na_3AlF_6 (mineral cryolite), or Na_3AlF_6 (synthetic cryolite)		15.0 mg/kg	Young pigs	168 days	Palpable osteofluorosis of mandible, and lesser degree of diaphyses

Source: Adapted from Cass, 1961, Table VI, p. 533. Data collected from several sources. Reprinted by permission of the publisher.

TABLE 5.28. THE EFFECTS OF ADDED DIETARY INCREMENTS OF FLUORIDE (AS SODIUM FLUORIDE) ON THE CALCIFICATION OF BONES AND JOINTS OF DAIRY COWS

Lot	Fluoride added (ppm)	Cow No.	Degree of osteofluorosis[a]		Degree of cartilage calcification[a]	
			Metacarpus	Metatarsus	Metacarpus	Metatarsus
I	0	2	0	0	0	0
		3	0	0	0	0
		4	0	0	0	0
II	20	5	0	0	1	1
		6	0	0	0	0
		7	0	0	0	0
		8	0	0	0	0
III	30	10	1	1	1	3
		11	0	0	1	1
		12	1	2	2	3
IV	40	13	1	2	2	2
		14	1	2	1	2
		15	1	1	0	1
		16	2	2	0	1
V	50	17	4	5	2	5
		19	3	3	2	4
		20	4	4	2	3
VI	50 + $CaCO_3$	22	4	4	1	4
		23	4	4	2	4
		24	3	4	1	2

[a] Based on a rating of 0 for normal bone and 5 for the most severely affected.

Source: Adapted from Suttie, Phillips, and Miller, 1958, Table 3, p. 300. Reprinted by permission of the publisher.

and enlarged and malformed bone trabeculae in young pigs fed 30, 60, and 90 days on a diet containing 1000 ppm sodium fluoride. Shorter and thicker bones were found in young pigs from sows fed a diet containing 450 ppm fluoride (Forsyth, Pond, and Krook, 1972). Decreased length, width, volume, and fresh weight of the humeri of baby pigs from sows fed additional fluoride indicated impaired skeletal growth of fetuses due to fluoride. At high fluoride levels, increased dietary calcium and phosphorus levels decreased fluoride accumulation in the offspring.

5.5.2.3.3 Lameness — Lameness associated with fluorosis is usually a nonspecific stiffness and is intermittent in nature (National Academy of Sciences, 1974). In advanced cases of fluorosis, lameness may be associated with bone lesions and calcification of ligaments. Lameness has been correlated with the degree of fluoride exposure (Table 5.29), but variation in other possible inducing factors makes a causal relationship difficult to confirm. In some severe cases, animals may eventually refuse to walk and stand, and intermittently move around on their knees (Shupe, 1972).

TABLE 5.29. LAMENESS ASSOCIATED WITH EXPOSURE OF LIVESTOCK TO ELEVATED FLUORIDE LEVELS

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure (mg/kg body wt)	Age at start of exposure	Duration of exposure before appearance of lesion	Effect on locomotion
Cattle	NaF in feed	50	Av ≃14 (peaks 2.0)	2 years of age, after first calving	At 5th lactation	Lameness and stiffness appeared for the first time
		100-200	Av 3.0-4.0	Yearling cattle	Within 8 to 16 months	Lameness and stiffness
	Raw rock phosphate in feed		Est. ~6.0		Common occurrence by 4th year	Lameness
	Natural fluoride in drinking water	18	≃2.0	Birth	During lifetime	Lameness
		25-32 or more			During lifetime	~20% of herd lame
	Pasturage contaminated with fluoride-containing atmospheric waste from industry	20-80		Young adults and adults	1-2 years	Lameness
		98		Adult dairy cattle	About 1 month	Lameness
Swine	Raw rock phosphate in feed	650		Weanlings	5-7 months	Reluctance to move and stiffness
	NaF in feed	580		Weanlings	144 days	Reluctance to move and stiffness
		1000		120-130 days old	40-75 days	Leg stiffness

Source: Adapted from Cass, 1961, Table VIII, p. 534. Data collected from several sources. Reprinted by permission of the publisher.

Cows fed a 93 ppm sodium fluoride diet showed signs of lameness at 2.5 years; after 3.5 years, two out of eight cows went down on their knees (Greenwood et al. 1964). After three weeks they partially recovered and apparently walked normally. However, intermittent lameness occurred again during the remainder of the trial period. Cows on 49 ppm sodium fluoride showed lameness after 4.5 years. It should be noted, however, that lameness in the absence of other signs of excessive fluoride ingestion cannot be considered an indication of fluorosis. Stiffness and lameness occur in livestock for reasons other than high fluoride ingestion.

5.5.2.3.4 Reproduction — There is some discrepancy in the literature concerning effects of excessive fluoride ingestion on reproduction. Most researchers agree that there is no direct effect on reproduction. However, because of fluoride's impairment of general body health, reproduction could be indirectly affected (National Academy of Sciences, 1974). Neeley and Harbaugh (1954) found that daily food and water levels of 0.52 to 1.69 mg of fluoride per kilogram body weight did not affect reproductive efficiency in cattle exposed for six years. Shupe (1972) and Shupe, Miner, Greenwood, Harris, and Stoddard (1963) also reported that reproductive efficiency in cattle was not affected when up to 93 ppm sodium fluoride (total dry-weight basis) was added to the diets. In contrast to these findings, cattle receiving 8 and 12 ppm fluoride in drinking water showed an increase in post-calving anestrus in the second season of reproduction (van Rensburg and de Vos, 1966). The third season revealed an appreciable decline in fertility in animals receiving over 5 ppm fluoride, and in the fourth season, cattle on 8 and 12 ppm also showed a reduced reproduction efficiency (Figure 5.12). This effect on reproduction was manifested prior to evidence of general health impairment. The authors suggested that the fluorine content of water should be under 5 ppm for normal reproduction since fluoride levels in water are more toxic than similar levels in dry matter.

5.5.2.3.5 General conditions and systemic effects — Excessive fluoride intake can result in appetite impairment, causing general unthriftiness and loss of condition (Shupe, 1972). Dry hair and thick nonpliable skin have been observed in animals with fluorosis. Fluoride effects can also be more severe on undernourished animals (Suttie and Faltin, 1973).

Stoddard, Harris, Bateman Shupe, and Greenwood (1963) reported that cattle receiving 93 ppm sodium fluoride in their diet consumed less hay and total dry matter than those on lower sodium fluoride levels. However, no significant loss in body weight gain was detected during the twenty-three 112-day periods of the trial. No effects were found on increases in height. Hobbs and Merriman (1962) and Hobbs et al. (1954) reported that cattle receiving rations containing 200 to 1200 ppm fluoride showed a decrease in body weight directly related to the level of fluoride ingestion. Table 5.30 presents the nutritional symptoms found with excessive fluoride exposure.

Sheep given water containing 10 ppm fluoride showed no adverse effects but did have a decrease in wool production (Peirce, 1959). Water containing 20 ppm fluoride adversely affected not only wool production but also the health of the sheep. Earlier, Peirce (1954) reported that intake of

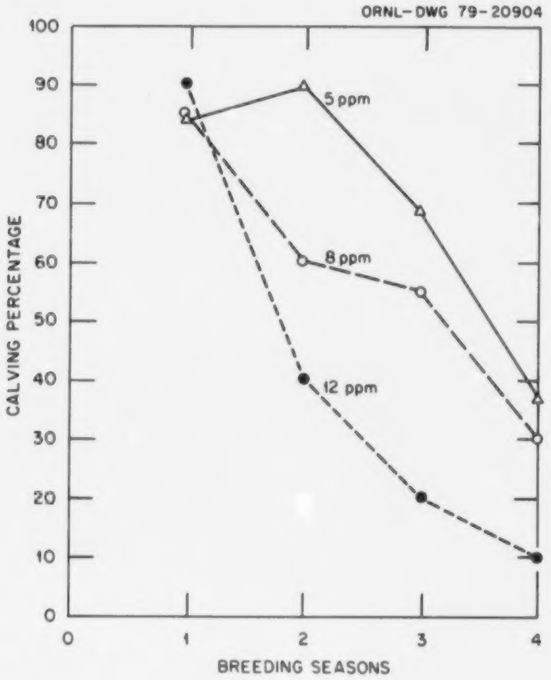

Figure 5.12. Calving rate of cows on three levels of fluorine intake. Source: Adapted from van Rensburg and de Vos, 1966, Figure 1, p. 189. Reprinted by permission of the publisher.

water containing up to 20 ppm fluoride had no effect on general health or body weight.

In evaluating fluoride effects on the hematopoietic system, liver, and thyroid gland in cattle, Hoogstratten et al. (1965) fed heifers 10, 25, 50, and 100 ppm sodium fluoride for 7.5 years. They found that concentrations up to 100 ppm did not induce gross, histological, or functional effects on the thyroid gland or liver. There were no significant changes in the serum calcium or phosphorus. In cattle fed 100 ppm sodium fluoride, a slightly higher total eosinophil count and a lower level of serum folic acid activity was induced. No anemia or abnormalities of the bone marrow were detected.

5.5.2.3.6 Milk production — Milk production is not directly affected by ingestion of low levels of fluoride. Effects on milk production are probably secondary to major symptoms and lesions and result from interference with lactogenesis and alteration of metabolic functions. Lactating cows were fed dietary fluoride (sodium fluoride) over a 5.5-year period. Under adequate nutritional conditions, 50 ppm fluoride had no effect on milk production. However, at 1.5 mg of fluoride per kilogram body weight, certain cows had reduced milk and butterfat production as a result of anorexia.

TABLE 5.30. NUTRITIONAL SIGNS ASSOCIATED WITH EXPOSURE OF LIVESTOCK TO ELEVATED FLUORIDE LEVELS

Animal	Source	Fluoride concentration in source (ppm)	Daily exposure (mg/kg body wt)	Age at start of exposure	Duration of exposure before appearance of lesion	Depressed feed intake	Depressed weight gains or growth
Cattle							
Beef	NaF in feed	$\geq$40	Av $\simeq$1.4	1-2 years	2 years or longer	Yes	Yes
Dairy	NaF in feed	40	1.4	Freshening 2 years old	5th lactation	Yes	No
		50	1.7	Freshening 2 years old	3rd lactation	Yes	Yes
		50	1.9	2nd and 4th lactation	3 additional lactations	No	No
		100	$\simeq$2.12	3 months	16 months	No	No
Hill bulls	NaF in feed		3.0	Adult	7 months or longer	*a*	Yes
Dairy	Rock phosphate in feed	440 and 880		117-193 days	During 1st lactation	Yes	Yes
Sheep							
Feeder lambs	NaF in feed	206		8-12 months	140 days	Yes	Yes
		106		8-12 months	140 days	No	No
		111		8-12 months	3 years	No	No
Lambs	Raw rock phosphate in feed		1.5	Growing	136-235 days	No	No
			3.0	Growing	136-235 days	No	*c*
			6.0	Growing	136-235 days	Yes	Yes
Ewes	Raw rock phosphate in feed		1.5-3.0	Yearling	2 years and more	No	*c*
			6.0	Yearling	2 years and more	Yes	Yes
Swine							
Self feeder in dry lot	Raw rock phosphate in feed	217		Young pig	2 years	*a*	Yes
		140		Young pig	2 years	*a*	No
		293	$\simeq$11 to 14	Young pig	2 years	Yes[b]	Yes
Self feeder in pasture	Raw rock phosphate in feed	293	$\simeq$11 to 14	Young pig	2 years	Yes[b]	No
Self feeder in dry lot	Raw rock phosphate in feed	569	$\simeq$19 to 26	Young pig	2 years	*a*	Yes
Self feeder in pasture	Raw rock phosphate in feed	569	$\simeq$19 to 26	Young pig	2 years	*a*	Yes

[a] Not mentioned.
[b] Sows during suckling.
[c] Slight.

Source: Adapted from Cass, 1961, Table VII, p. 534. Data collected from several sources. Reprinted by permission of the publisher.

Cows tolerated 30 ppm fluoride, 40 ppm fluoride was marginal, but 50 ppm induced fluorosis. A latent period of two to five years elapsed between initial dental effects and inhibition of milk production. Stoddard, Bateman, Harris, Shupe, and Greenwood (1963) reported that 93 ppm fluoride reduced milk production in the third and subsequent lactations of Holstein cows maintained on diets with added fluoride from three months of age to 7.5 years of age. Milk production of some cows on 49 ppm sodium fluoride was adversely affected in the fourth and subsequent lactations. The tolerance level of fluoride for lactation was less than 49 ppm on a total dry-diet basis (Stoddard, Bateman, Harris, Shupe, and Greenwood, 1963).

5.5.2.4 Tolerance — Tolerance to fluoride varies with the solubility of the fluoride in the gastrointestinal tract (Phillips, 1956). At levels of fluoride where fluorosis occurs, there is a difference in toxicity of soluble and relatively insoluble fluorides. Therefore, tolerance levels for soluble fluorides and fluorides exposed by water solutions are lower than the tolerance levels for fluoride in dry matter (Underwood, 1971).

Fluoride tolerance differs among species, as shown in Table 5.31. Horses are able to tolerate more fluoride than beef or dairy cattle but less than feeder lambs. Even though Table 5.32 indicates cattle as being the most sensitive of livestock, Peirce (1959) has shown that sheep have adverse effects from fluoride concentrations in water as low as 20 ppm. Swine appear to be the most resistant mammal to fluorosis. Breeding and lactating animals seem to have a lower tolerance for fluorides than do finishing animals (Table 5.32). This is partially due to both the eventual human utilization of the animal and the exposure length — dose response patterns associated with these uses. For example, Harris et al. (1958) demonstrated that total feed levels of 112 ppm fluoride (dry weight) had no significant effect on the performance of feeder lambs over a fattening period of 14 weeks.

Therefore, establishment of tolerance levels for livestock should take into consideration the economic use and well-being of the animal. Animals used for breeding purposes have a lower tolerance than those being used for slaughter, since the long-term general health of the animal influences breeding success.

TABLE 5.31. DIETARY FLUORIDE TOLERANCES FOR DOMESTIC ANIMALS[a]

Animal	Performance[b] (ppm)	Pathology[c] (ppm)
Beef or dairy heifers	40	30
Mature beef or dairy cattle[d]	50	40
Finishing cattle	100	NA[e]
Feeder lambs	150	ID[f]
Breeding ewes	60	ID
Horses	60	40
Finishing pigs	150	NA
Breeding sows	150	100
Growing or broiler chickens	300	ID
Laying or breeding hens	400	ID
Turkeys[g]	400	ID
Growing dogs	100	50

[a] Values are presented as ppm fluoride in dietary dry matter and assume the ingestion of a soluble fluoride, such as sodium fluoride. When the fluoride in the ration is present as some form of defluorinated rock phosphate, these tolerances may be increased by 50%.

[b] Levels fed without clinical interference with normal performance.

[c] At this level of fluoride intake pathologic changes occur.

[d] Cattle first exposed to this level at three years of age or older.

[e] NA — not applicable.

[f] ID — insufficient data.

[g] Level shown to be safe for growing female turkeys.

Source: Adapted from the National Academy of Sciences, 1974, Table 4, p. 55. Reprinted by permission of the publisher.

TABLE 5.32. TOLERANCE OF ANIMALS FOR FLUORIDE[a]

Species	Fluoride concentration in ration (ppm)	
	Breeding or lactating animals	Finishing animals to be sold for slaughter with average feeding period
Dairy and beef heifers	30	100
Dairy cows	30	100
Beef cows	40	100
Steers	NA[b]	100
Sheep	50	160
Horses	60	NA
Swine	70	ND[c]

[a] Tolerances based on sodium fluoride or other fluorides of similar toxicity.

[b] NA — not applicable.

[c] ND — not determined.

Source: Adapted from Shupe, Olson, and Sharma, 1972, Table 1, p. 358. Reprinted by permission of the publisher.

SECTION 5

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SECTION 6

BIOLOGICAL ASPECTS IN HUMANS

6.1 SUMMARY

Fluoride at an intermediate concentration (biological verging to pharmacological) is beneficial to humans, but high concentrations are toxic. Fluoride may be essential for nucleation of bone crystalline material, maintenance of reproduction, and other metabolic processes.

There is continuing controversy engineered by those opposing fluoridation of water as a public health measure. Evidence is overwhelming that fluoridation is beneficial and is the most cost-effective and safest way of improving dental health in a population. An even higher level of fluoride than the 1.0 ppm considered optimal for caries resistance may be of benefit to older persons in preventing osteoporosis. Thus there appears to be no reason to withhold the benefits of fluoridation from the general population. Despite controversial aspects of the problem, it appears the real obligation of a public health authority is maintaining an optimal level of fluoride in the public water supply, adding fluoride when it is lacking and removing it when there is excess.

Man's intake of fluoride is chiefly through the diet with drinking water furnishing the largest amount. Absorption of fluoride is largely passive; the amounts absorbed depend on solubility. Absorbed fluoride is quickly distributed throughout the body, easily crossing membranes and going into tissues. Bone is a sink for fluoride, and uptake is so effective that bone blood flow can be measured by the rate of scavenging of ^{18}F from blood. Excess fluoride is sequestered by bone and released slowly when the intake level subsides. Bones primarily accumulate fluoride as fluorapatite, and to a lesser extent as magnesium fluoride, throughout an individual's lifetime. The pattern of fluoride accumulation versus the age of an individual is not fully known. Long-term intake of fluoride at levels above 8 ppm causes fluorosis. Fluorosis is chiefly manifested by changes in the bony structure with some parts of the bone becoming hypercalcified and other parts showing deterioration. In radiographs, these bones are more dense and less structured than normal bones. When fluorosis is pronounced, exostoses and bony infiltrations may cause crippling and pain. Once in the bone, fluoride does not cause fluorosis; damage occurs when fluoride is present in excess and interferes with the metabolism of the osteocytes and the osteoblasts.

Fluoride absorbed into blood is excreted largely through the kidneys, by relative lack of reabsorption. Some fluoride is excreted in sweat, and there is a slight secretion through the mucosa of the intestines into the gut. Some of the fluoride in blood is organically bound, which may represent uptake of commercially used fluorinated products. The amounts are small, and there is no indication of a hazard.

The fetus obtains the fluorine needed for calcification of bony tissues from its mother. The placenta plays a largely passive role but may sequester fluoride through calcifications in case of excess.

Fluoride (at concentrations greater than trace level) inhibits some enzymes including oxidative enzymes, phosphate-transferring enzymes, and some lipases. It often acts by interfering with the magnesium necessary for the enzyme action. Although fluoride stimulates adenylate cyclase, the significance of this behavior is not clear because the effect has been demonstrated primarily in broken cell preparations. At levels normally found in blood, fluoride does not seem to have any deleterious effect on any particular organ, including the thyroid gland; however, it can adversely affect a number of physiological processes such as nerve action, protein synthesis, membrane permeability, and possibly hormonal secretions.

Fluoride is an irritant poison and is lethal starting at oral doses of about 3 g. Aspects of fluoride poisoning leading to death are: blockage of respiratory enzymes; interference with necessary body functions controlled by calcium, such as blood clotting, membrane permeability, nerve transmission, and muscle action; specific organ damage, particularly to the kidneys; shock; and general collapse. Except for organ damage, the effects are reversible, and with prompt treatment not only can a person survive but recovery can be complete. In the case of exposure to hydrogen fluoride and F_2, the extremely corrosive nature of the reacting compounds adds further damaging effects to the general fluoride toxicity. Absorbed as vapors, they cause extreme irritation to the lungs resulting in edema and tissue damage, as well as the systemic toxic effects.

Although the public may be exposed to numerous inorganic- and organic-containing products, concentrations of these products are generally low and innocuous. Man's chief exposure to excessive fluoride occurs in industrial situations, as in processing industries where fluoride is associated with other substances (e.g., phosphate fertilizer processing) or where fluorides are being used as part of some process (e.g., steel making or in chemical industries). Threshold limit values for industrially encountered fluoride compounds have been established. Exposure levels may be monitored by air sampling, and worker uptake may be monitored by urine analysis.

Information presented in this chapter indicates that inorganic fluoride is probably not carcinogenic, but that at high doses it may be mutagenic and teratogenic in experimental animals. The results of one study, however, indicate that fluoride is not mutagenic in mice or bacteria. It is important to realize that the concentrations of inorganic fluoride normally encountered by the general public (e.g., in fluoridation of public water supplies) are not teratogenic, mutagenic, or carcinogenic. There is concern, however, about potential teratogenic and other reproductive effects among persons chronically exposed to fluorinated anesthetics (organic fluoride compounds).

6.2 ESSENTIALITY OF FLUORIDE

Because of the ubiquitous occurrence of fluoride in its various forms and the obviously long association of living things with fluoride-containing compounds, researchers have repeatedly tried to test whether fluoride is an essential element for life. A representative study was done by Doberenz et al. (1964). The difficulty in these studies is in eliminating fluorine from the diet. The researchers prepared a diet from hydroponically grown sorghum and soybeans that contained only 0.005 ppm fluoride. Amino acid analyses of the soybeans and sorghum after several generations of growing with very low concentrations of fluoride were no different from those of plants grown under field conditions. The diet, with and without added fluoride, was fed to rats from weaning to 90 days of age. Growth of the rats was not impaired, and of numerous serum and liver enzyme activities tested the only significant differences noted were an increase in serum isocitric dehydrogenase and a concomitant decrease in activity of this enzyme in the liver. Levels of bone fluoride were significantly lower, but no adverse physiological effects were noted. Thus, essentiality was not demonstrated.

Messer, Armstrong, and Singer (1974) also reviewed the question of essentiality. They established two criteria as minimum requirements that must be met before fluorine, or any other trace element, was considered essential: (1) a specific deficiency state should be produced by a diet lacking the element in question, but which is otherwise adequate and satisfactory; and (2) the deficiency should be prevented or cured by addition to the diet of that element alone. They added to these requirements three supporting criteria: (1) in general, the deficiency should be correlated with subnormal tissue levels of the element; (2) the deficiency should be accompanied by pertinent biochemical or physiological changes which will be prevented or cured when the deficiency is prevented or cured; and (3) a homeostatic control mechanism regulating tissue levels of the element should be demonstrable.

On the basis of studies with mice and rats showing retarded growth, infertility, or anemia on low-fluoride diets and subsequent recovery from these states upon administration of fluoride, Messer, Armstrong, and Singer (1974) felt that their first two criteria had been met and that fluorine was an essential trace element; however, specific biochemical lesions were not detected, nor were biochemical mechanisms underlying the conditions defined.

Fertility and growth may be affected by lack of fluoride. Maurer and Day (1957) maintained reproduction in rats over four generations with a diet estimated to contain only 0.007 ppm fluoride. They found that the viability of the offspring was impaired. In a study by Messer, Armstrong, and Singer (1972), female mice maintained on a low-fluoride diet showed a progressive decline in litter production over two generations. The decreased fertility may have been the result of an irregular estrous cycle or a slowing of the onset of sexual maturity, implying that hormonal factors could have been involved. Fertility, however, was restored when the diet was supplemented with fluoride. Bone-ash (humerus) fluoride content fell, but no adverse effects were noted. The authors concluded that the infertility, which took several generations to manifest itself, constituted a deficiency state produced by a diet apparently otherwise nutritionally adequate. This conclusion must be examined more critically because fluoride has been shown to have manifold effects on cells and cell systems.

In contrast to the studies of Messer, Armstrong, and Singer (1972, 1974) which suggested that fluoride was essential for reproduction in the mouse, Tao and Suttie (1976) conducted reproduction studies in mice and found that restriction of maternal fluoride intake did not significantly influence reproduction rate, litter size, or litter weights. The reproduction of mice fed a basal diet of <0.5 ppm fluoride was the same as when the basal diet of the mice was supplemented with 2 or 100 ppm fluoride. Although the fluoride levels in the basal diets of the mice were essentially the same in the experiments of Tao and Suttie and of Messer, Armstrong, and Singer, the iron and copper levels were not the same. The diets of the mice in the Messer, Armstrong, and Singer experiments were deficient in iron and copper levels which Tao and Suttie suggested was the cause of the impaired fertility; the addition of fluoride prevented this deficiency through more efficient utilization of the dietary levels of iron and copper. Thus, the role of fluoride on the reproduction of the mice was secondary. In support of this hypothesis, Tao and Suttie (1976) sited the works of Ruliffson, Burns, and Hughes, where fluoride was shown to enhance intestinal iron absorption in rats, and of Hamuro where fluoride prevented magnesium deficiency in mice.

Schwarz and Milne (1972) considered the effects of other trace elements (particularly tin, vanadium, and silicon) and showed that fluorine, supplied as potassium fluoride, was essential for the growth of rats that were kept in isolators and fed highly purified amino acid diets. The authors emphasized that the amounts of fluoride required for growth and development were physiological. Fluoride levels in human diets not deficient in fluoride are similar to those that maintained growth of the rats in the study.

Mechanisms of fluoride action are elusive. Potentiating effects on enzymes and possible involvement with the action of hormones have been mentioned. In the studies of Messer, Armstrong, and Singer (1974) in which anemia conditions were noted, it was suggested that fluoride may have had something to do with either the uptake or utilization of iron (see also Tao and Suttie, 1976, in this section). Fluoride is involved in the metabolism of calcifying tissues and has been described by Hodge and Smith (1972) as the "prototype bone-seeker." Conversely, bone reacts with fluoride, displacing the hydroxyl and carbonate groups of the bone material. It has been suggested that fluoride is essential for nucleating the precipitation and crystallization of hydroxyapatite in the formation of bone (Newesely, 1961; Perdok, 1962). The concentrations required may be low. Brown (1966) calculated that fluoride concentrations in the range of 0.0002 to 0.002 ppm should be sufficient for the process of promoting the transformation of octacalcium phosphate to fluorohydroxyapatite with subsequent crystallization. Although a requirement for fluoride in calcification probably exists, the difficulty of achieving such low concentrations in work with animals may prevent rigorous experimental demonstration of the need.

Fluoride is probably needed for the maintenance of bony structure. Underwood (1971) suggested that the high incidence of osteoporosis in elderly persons, especially women, could be regarded as a fluoride deficiency. Fluoride concentrations required to prevent osteoporosis are somewhat higher than those considered optimal for development of caries-resistant teeth in children, thus complicating the public health management of fluoride.

It is generally thought that of all the essential trace elements, fluoride has the narrowest margin between beneficial and undesirable concentrations. In a well-balanced review of criteria for the essentiality of trace elements, Mertz (1970) clearly showed the different levels of response of biological systems to trace elments: a biological response level (e.g., catalytic effects, enzyme element requirements, and synergistic effects), a pharmacological level (resistance to caries, possible prevention of osteoporosis, and possible inhibition of aortic calcification), and a toxic level. These ranges are often distinguished by plateaus of responses, before a new range is attained. Mertz pointed out that where the plateaus between ranges are narrow, or where the ranges merge, is an indication that homeostasis is inefficient or for some reason hindered. This may be the case with fluoride. Beyond excretion, which is passive, and uptake by bone, a real homeostatic mechanism for shutting out excess fluoride does not seem to exist. Toxic effects result from acute and chronic exposure to excessive fluoride concentrations. From a biological standpoint, the problem of evolving control is difficult because the ranges are so narrow.

In summary, some evidence indicates that fluorine may be essential for man and other higher animals (Mertz, 1970; Schwarz, 1974); however, exact mechanisms remain uncertain. More than most trace elements, fluorine shows a closeness among physiological (essential), pharmacological (beneficial and preventive), and toxic dose ranges.

6.3 METABOLISM

6.3.1 Uptake

6.3.1.1 Entry — Except in polluted and occupational environments where fluoride compounds are inhaled as vapors, fumes, or dusts, humans are exposed to fluoride mainly through food and beverages. In modern societies where extensive food processing is the rule, any fluoride-containing residues will generally have been washed off the food. Thus, intake is mainly from fluoride in the food itself. Jones, Harries, and Martin (1971) measured the amounts of fluoride found in or on leafy vegetables grown in England near sources of atmospheric pollution by fluorides, both particulates and gases. Their studies showed that even when vegetables were poorly washed, increased intake of fluoride was not significant. Some fluoride was supplied by vegetables, but fluoride intake from water was more important.

6.3.1.2 Absorption — Fluorine in food and water is mainly in the form of fluorides. In foods, fluoride may be complexed with a counterion such as calcium or aluminum, rendering it insoluble. This complexing decreases, but does not entirely prevent, the absorption of fluoride. The absorption of soluble fluoride is very rapid, starting in the stomach. In a study by Carlson, Armstrong, and Singer (1960*a*) a small dose of radiolabeled fluoride was given orally to human volunteers. A rapid decline in radioactivity was noted in the region of the stomach only a few minutes after ingestion. Animal experiments using isolated sections of the gastrointestinal tract have also shown rapid absorption of soluble fluorides; the chief region of absorption in both man and animals is the upper part of the small intestine.

Absorption of fluorides seems to be a passive process. Excretion of fluoride into the mucosa of the intestines may, however, be an active transport process. In studies with several species of rodents, Parkins (1971) showed active transport of fluoride in a secretory direction across everted sacs of intestinal sections. A decrease in the rate of fluoride transport was observed with increased age of the animals. The authors correlated the reduced transport with the fairly low base levels of fecal excretion of fluoride found by Spencer et al. (1969) in a study of the effect of fluoride supplementation on absorption of calcium in middle-aged patients.

The chemical form of fluorine and the presence of other factors influence its absorption. Thus, the absorption of fluoride from ingestion of products containing bone is hindered: for instance, only about 50% of the fluoride from fish protein concentrate is absorbed because most of the fluoride is in the form of the highly insoluble fluorapatite. On high-fat- and high-cereal-product diets, absorption of fluoride is slowed. The presence of milk and milk products in the diet also slows absorption of fluoride. Milk contains very little fluoride, and even high levels of fluoride in the diet do not result in the appearance of much fluoride in the milk of lactating animals. Since the need for supplementing the diet with fluoride for caries prevention is greatest in children, it has been suggested that fluoride be added to milk rather than drinking water. Further, since many areas of the world are not served with fresh milk, it would be convenient to add the fluoride to dried milk. Konikoff (1974) studied the availability of fluoride as a function of time in reconstituted dried milk (with and without additions of calcium) and found that almost all of the fluorine remained available in the ionic state up to 4 or 5 hr. After this time, about 20% of the initial fluoride occurred as CaF_2 and was therefore not readily available. There was then a slow decline in fluoride availability over six to seven days. Absorption of fluoride from milk was slower than from water, as shown by the fact that while 1 ppm fluoride added to fresh milk could be detected in the urine in about 90 min, it was detected in 30 min when added to water.

6.3.1.3 Appearance in Body Fluids — Absorption can be measured indirectly by the appearance of fluoride in some easily accessible body fluids (e.g., blood, urine, or saliva). In the study by Carlson, Armstrong, and Singer (1960*a*), human subjects were given 1 mg of stable fluoride labeled with radiofluoride in water, preceded and followed by water over the observation period. Blood, saliva, and urine samples were taken. Maximum plasma radiofluoride concentration was reached in 60 min. At that time, the concentration in the urine was increasing. It reached a maximum at about 240 min. The appearance and decline of radiofluoride in the saliva paralleled those in the blood, at a slightly lower concentration level; however, because of the difference in volume, less than 1% of the ingested activity was recovered in the saliva. Fluoride-urine clearance was at all times manyfold greater than the chloride clearance. A large urinary clearance of fluoride was maintained even when the urine volume decreased. Readings with a crystal scintillator probe over various body areas easily followed gastric emptying and dissemination of the fluoride in the soft tissues and into bone. Skeletal tissues retained the isotope. For instance, whereas counts over the biceps had declined to nearly zero at 250 min after ingestion, counts over the femur had decreased by only 15% from the maximum value.

Brudevold, Bakhos, and Gron (1973) studied the appearance of fluoride in saliva after ingestion of sodium fluoride or sodium monofluorophosphate, alone and with additions of varying amounts of aluminum chloride. Fasting adults were given 5 mg of fluorine as sodium fluoride or Na_2PO_3F in a gelatin capsule, washed down with 10 ml of water. The capsules contained either no aluminum or 3.5, 7.11, or 14.2 mg aluminum as aluminum chloride. After ingestion of fluoride without aluminum, the salivary fluoride increased rapidly, peak levels of about 0.2 ppm being reached in about 50 min. With the aluminum additions, the rates of increase of fluoride in the saliva were decreased, roughly in proportion to the amount of aluminum. The peak levels were also lower. Since no aluminum was found in the saliva, it was felt that fluoride uptake was hindered at the level of absorption in the digestive tract by aluminum complexing with fluoride, and not by an effect at some later stage such as passage of fluoride from the blood to the saliva.

When fluorine was given covalently bound as sodium monofluorophosphate, Brudevold, Bakhos, and Gron (1973) found that the rise of salivary fluoride was slower in all subjects and the peak levels lower in three out of five than when ionic fluoride was given. Response to the aluminum additions was similar to the case of ingestion of fluorine as fluoride. Only ionic fluorine was detected in the saliva, indicating that the fluorine of the monofluorophosphate was hydrolyzed and then absorbed as fluoride.

An interesting result from the studies with the aluminum additions is that while rates of increase of salivary fluoride were lowered and peak levels were lower, rates of decline of fluoride concentration were also lowered in some cases. Thus, Brudevold, Bakhos, and Gron (1974) postulated that aluminum intake within a certain range might actually increase rather than reduce fluoride utilization and play a quasi-regulatory role, particularly with respect to the effect of fluoride on teeth.

Sodium monofluorophosphate is one of the fluorine compounds added to toothpaste for prevention of caries. Aside from the expected topical effect, some of the compound may be swallowed. Glass et al. (1975) studied fluoride ingestion resulting from the use of a monofluorophosphate dentifrice by children. The amounts of fluoride absorbed were minor with respect to the total fluoride intake and presented no danger of excess fluoride even in areas of fluoridation. A similar conclusion was reached by Duckworth (1964) studying the distribution and excretion of dentifrice stannous fluoride. Amounts absorbed caused urinary excretion of fluoride amounting to only 5% to 10% of the usual daily physiological variation.

6.3.1.4 Potential Absorption of Fluoride from Tobacco Smoke — Along with data on the fluoride content of a variety of environmental agents (e.g., detergents and other cleaning materials, dusts, pollen grains, and fertilizers), Waldbott and Oelschlager (1974) gave figures for the fluoride content of several brands of cigarettes and one brand of cigar. These ranged from 17.0 ppm for one German cigarette to 19.2 for a German cigar, 21.7 and 22.1 ppm for two Austrian cigarettes, 24.2 and 26.8 ppm for two other German cigarettes, and an average of 25.0 ppm for several U.S. brands. A heavy smoker (50 cigarettes a day) inhales approximately 0.8 mg of fluoride (this number is not greater because about 80% of the cigarette is consumed and not all the smoke is inhaled) which is not an inconsiderable amount in view of the usual normal intake of 2 to 3 mg a day. Waldbott and Oelschlager (1974) suggested that fluoride through its association with such materials as asbestos and fiberglass could be a factor in the irritating action of these agents. These aspects are considered in Section 6.4.3.3, along with a discussion on the effects of compounds of fluorine other than simple fluorides.

6.3.2 Distribution and Balance

6.3.2.1 Excretion — Fluoride from soluble fluoride compounds is rapidly absorbed into the blood and appears with only slight lag in the urine (approximately 90% of that absorbed) and saliva. Fecal fluoride is often reported as 5% to 10% of total intake; however, the proportion varies depending on circumstances. Table 6.1 shows balances between urine and stool under controlled conditions, during supplementation with sodium fluoride, and following supplementation (Spencer et al., 1970). The average intakes of 4.39 and 4.23 mg of fluoride per day from the institutional diet, including drinking water given before and after supplementation, are higher than the 2 mg/day which is the typical target concentration when water is fluoridated. Noteworthy in the study was the relatively constant urinary excretion of fluoride in successive study periods of the control groups. On forcing with fluoride, the balance rose to an average of 5.4 mg/day, the excess fluoride presumably depositing in bone. Retention was high, 42.9% of intake in the control period and 39.3% during supplementation, returning to 31.9% in the period after supplementation.

Figure 6.1 shows the great rise in urinary excretion of fluoride following supplementation. Not all fluoride given was excreted, the balance going to bone. On discontinuation of supplementation, excretion fell rapidly to only a few percent of the retained fluoride, then fell slowly with time.

TABLE 6.1. FLUORIDE BALANCES BEFORE, DURING, AND AFTER SODIUM FLUORIDE (NaF) SUPPLEMENTATION

Patient	Fluoride (mg/day)											
	Control				NaF				After NaF			
	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance
1	3.93	1.84	0.19	+1.90	13.27	7.59	0.59	+5.09	3.59	2.43	0.26	+0.90
2	3.57	1.51	0.19	+1.87	13.14	6.01	0.63	+6.50	3.65	1.92	0.21	+1.52
3	4.47	2.41	0.27	+1.79	13.46	7.71	0.62	+5.13	5.00	3.45	0.60	+0.95
4	5.37	3.09	0.40	+1.85	15.09	9.27	0.95	+4.87	5.31	3.39	0.37	+1.55
5	4.47	2.09	0.19	+2.19	14.03	6.94	0.58	+6.61	4.31	2.45	0.21	+1.65
6	4.56	2.30	0.38	+1.88	14.26	8.12	1.56	+4.58	4.46	2.78	0.47	+1.21
7	4.11	1.91	0.26	+1.94	13.56	6.60	1.11	+5.85	3.87	2.15	0.33	+1.39
8	4.50	2.56	0.37	+1.57	13.67	7.79	1.25	+4.63				
9	4.29	2.30	0.25	+1.74	13.70	7.90	0.48	+5.32	4.07	2.35	0.24	+1.48
10	4.54	2.51	0.43	+1.60	13.20	6.85	0.98	+5.37				
Av	4.39	2.26	0.29	+1.84	13.79	7.52	0.88	+5.39	4.23	2.56	0.32	+1.35

Source: Adapted from Spencer et al., 1970, Table VI, p. 811. Reprinted by permission of the publisher.

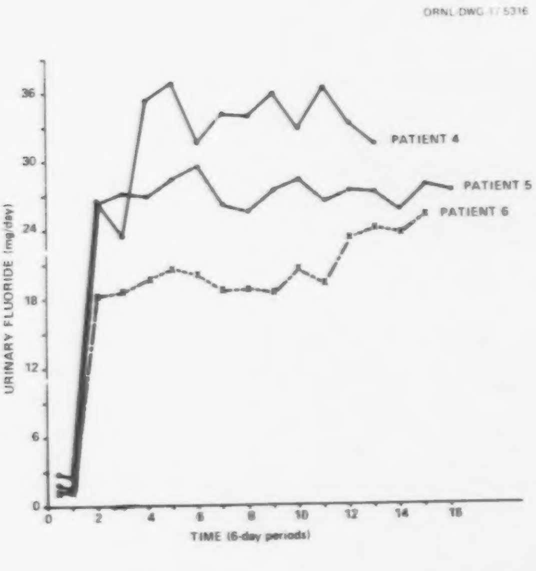


Figure 6.1. Urinary fluoride excretions during fluoride supplemental intake of 45 mg/day. Source: Adapted from Spencer, Osis, and Wiatrowski, 1975, Figure 2, p. 615. Reprinted by permission of the publisher.

Largent (1961) showed that the retention and storage of fluoride in the human body is dependent on such factors as the chemical form of the fluoride and the size and duration of the dose. Aqueous solutions of calcium fluoride (6 mg) and sodium fluoride (6 mg) were almost completely absorbed, whereas undissolved calcium fluoride (6 mg) and bone meal (6 mg) were only partially absorbed with 30% and 63%, respectively, of that ingested being found in the feces. One substance, fluoborate (6 mg), was unique in that all of the fluoride that was absorbed into the blood was excreted and recovered in the urine. For all the other materials tested (6 mg each), slightly less than 50% of the absorbed fluoride was recovered in the urine with the other 50% of that absorbed apparently stored in the tissues; a small percentage of that assumed to be stored possibly was lost through the skin. Largent (1961) conducted further observations of fluoride ingestion at levels of 12, 18, 19, 25, and 36 mg/day and found the results to be essentially the same — slightly more than 50% of the fluoride absorbed apparently was stored in the bone tissues.

In another series of experiments with an adult human subject, Largent (1961) administered extra dietary fluoride (sodium fluoride) at a dose of 3 mg/day for 25 weeks, and sometime later 12 mg/day was administered for 130 weeks. The rates of storage were plotted and a calculated curve with the equation of $y = 800(1-e^{-0.043t})$ was used to make predictions of skeletal fluoride saturation. At a dose of 12 mg/day, the skeletal tissues approached a 95% dose-level saturation in approximately 70 weeks, a 97% level in about 100 weeks, and a 99.8% saturation level in approximately 200 weeks. A steady state of balance between intake and output of fluoride would be reached sometime between 1.5 and 3.0 years after the start of ingestion of 12 mg/day. If the amount of fluoride ingested was increased above 12 mg/day then the rate of storage would abruptly increase and then gradually decrease until a new state of equilibrium was reached.

Fluoride is excreted more efficiently than chloride by being less efficiently reabsorbed. The process is one of glomerular filtration with a variable amount of tubular reabsorption. At normal charges of chloride and fluoride, renal resorption of chloride is above 99% compared with about 92% for fluoride (Deichmann and Gerarde, 1969). Urine flow rate and chloride clearance were considered the main determinants of fluoride clearance, but in recent experiments with pentobarbital-anesthetized rats, Whitford, Pashley, and Stringer (1976) were able to dissociate these factors from fluoride clearance. Their results suggested a pH dependence of fluoride renal clearance, tubular reabsorption of fluoride being inversely related to tubular pH. The fluoride reabsorption seemed to occur by nonionic diffusion, apparently as hydrogen fluoride. These findings also have application to absorption of fluoride from the stomach.

Sweat contains some fluoride and can be a significant route of excretion among highly active workers and in hot climates. To some extent it may compensate for higher intake of fluoride from drinking water. As much as one-third of a small dose of fluoride has been recovered in sweat (Hodge and Smith, 1970), and McClure et al. (1945) noted figures of 13% to 38% of ingested fluoride appearing in sweat under conditions of controlled temperature and humidity. Usually only a few percent of the normal intake of fluoride is excreted in sweat. The bulk appears in urine, but nonabsorbed and some secreted fluorides are found in the intestinal excretion.

6.3.2.2 Deposition and Distribution — Along with urinary excretion, uptake by bone effectively removes excess fluoride. On low and moderate fluoride intakes, there is a slow accumulation of fluoride in the bone, ultimately with the renewal rate equal to the deposit rate, and a steady state between intake and elimination at any one time (Hodge and Smith, 1970, p. 102). Figure 6.2 shows fluoride accumulation with age in bones at different long-continued levels of intake. Although this figure shows a plateau with respect to fluoride accumulation, other data do not show this pattern. The results of a study (Weatherell, 1966) on the fluoride content of femoral compacta from humans of different ages who lived in areas supplied with drinking water containing less than 0.5 ppm fluoride showed that accumulation was more or less linear regardless of age. There is obviously a need for further study regarding the accumulation of fluoride versus age.

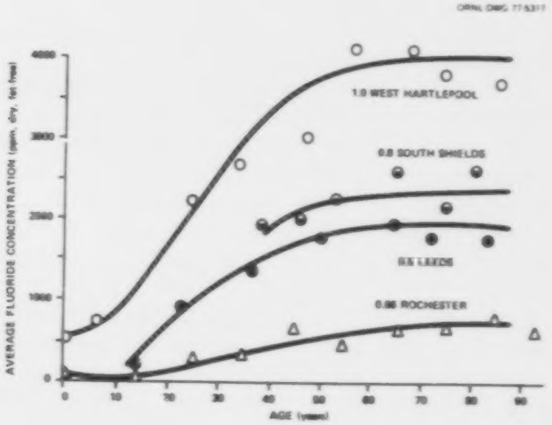


Figure 6.2. Skeletal concentrations of fluoride in residents of West Hartlepool, South Shields, and Leeds, England, and Rochester, New York. Adapted from Hodge and Smith, 1970*a*, Figure 2, p. 104. Reprinted by permission of the publisher.

The deposition of fluoride in bone shows two main phases. The first phase, which may be quite rapid and may result in a high concentration of fluoride in surface bone layers, results from perfusion of extracellular fluid containing fluoride into the osteocyte lacunae and the bone canaliculi (Gedalia and Zipkin, 1973). The fluoride ions displace hydroxyl groups in the bone crystal to make a mixed fluorohydroxyapatite (Hodge and Smith, 1970). Florkin and Stotz (1971) estimated that 1% to 2% of the skeleton is available for this exchange and that the rate and amount of uptake of a given dose depends on previous exposure to fluoride.

The second phase of fluoride deposition is in the denser parts of the bone. The interlacunar crystals of fully calcified bone are so closely packed as to largely exclude fluid (Rich and Feist, 1970). Thus, the diffusion of ions to this part of bone is impeded. Nonetheless, during mineralization and through turnover, fluoride is incorporated into these crystals at a rate paralleling the deposition of phosphorus as bone phosphate. The substitution of fluoride for ions in the crystal surface is termed by Weidmann and Weatherell (1970, p. 109) as "exchange," and incorporation into the crystal lattice is termed "uptake by accretion." Both processes are maximal during the phase of bone growth or tooth development when crystallites are forming and are still small, thus offering greater surface in proportion to their mass. In addition, young mineralized tissue has a high degree of hydration, which ensures high permeability and good access of tissue fluids to crystal surfaces.

Neither the fluoride deposited in surface layers nor that in the deeper crystals of bone is irreversibly fixed, in spite of the low solubility of fluoroapatite. Fluoride in the surface bone is in equilibrium with fluoride in the extracellular fluid (Florkin and Stotz, 1971). On deprivation, fluoride is mobilized, partly from back exchange of fluoride from crystal surfaces and partly from dissolution of crystals in the osteoclastic-osteoblastic cycle of bone remodeling (Hodge and Smith, 1970). Mobilization of fluoride (i.e., excess of excretion over intake following storage) is shown in Figure 6.3. The "removal half-life" of fluoride is eight to ten years (Forbes et al., 1978).

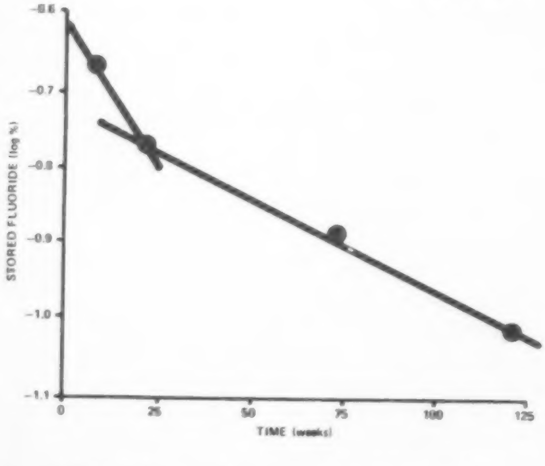


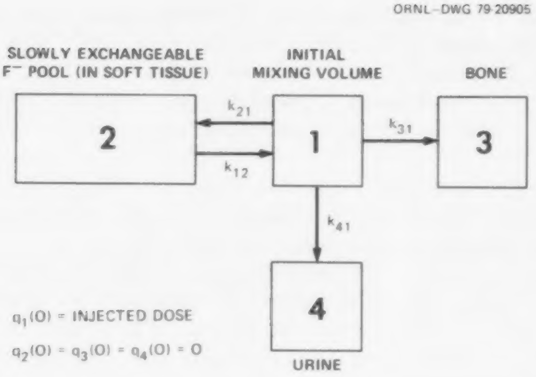
Figure 6.3. Mobilization of fluoride from the human skeleton. Adapted from Hodge and Smith, 1965, Figure 64, p. 543. Reprinted by permission of the publisher.

Bone is a sink for fluoride, and the ultimate capacity for storage of fluoride as fluorapatite is great. Hodge (1964) calculated that the theoretical limit is of the order of 35,000 ppm. Fluorosis, however, would appear long before this level was reached (Section 6.4.2.1). Figure 6.2 shows that a level of 4000 ppm was reached on a long-term intake of 1 ppm fluoride. At an intake level of 1.5 to 2.0 mg/day the long-term bone level reached 5000 ppm (American Academy of Pediatrics, 1972). Up to an intake level of 3 mg/day, no adverse effects were noted, except for tooth mottling and possible mild fluorosis of the bones. At levels of 10 to 15 mg/day or more, which may be reached under conditions of high industrial contamination, cases of skeletal fluorosis were noted (Largent, 1961). Osteosclerosis, or hypercalcification, may be detected by increased opacity of the bones to X rays at a deposition level of 6000 ppm (National Academy of Sciences, 1971). It has been estimated that a continuous intake of 8 ppm (the drinking water fluoride level that persisted for several years in Bartlett, Texas) in the diet for 35 years would be necessary for this level to be reached.

Fluoride uptake and concentration differ among bones and parts of bones. In the long bones, concentration of fluorine is lowest in the shaft and higher at each end as a result of growth and remodeling (Hodge and Smith, 1970). Bones with a tendency to exostosis formation, such as the shaft bones and mandibles, have a high affinity for fluoride. Fluoride in the exostoses can be as much as 20 times as high as in other parts of these bones under conditions of high fluoride intake (Underwood, 1971). Fluoride is incorporated more rapidly into surface (periosteal and endosteal) regions of bones than in the middle regions (Weidmann and Weatherell, 1959), and more rapidly into cancellous (spongy) than into cortical (compact) bones and parts of bones. Bones that have a high cancellous component (e.g., ribs, vertebrae, and sternum) accumulate a higher level of fluoride than cortical bones (Marier, Rose, and Boulet, 1963). Hefferren et al. (1972) studied the fluoride distribution in wedge and needle biopsies of iliac crest samples taken at necropsy from control and uremic patients and found that the average fluoride content of the control samples was 800 ppm and of the uremic samples, 2000 ppm, reflecting the poor excretion of the uremic state. In all samples, an increasing fluoride gradient of about 25% was noted from the surface to a depth of 8 mm or more into the bone; fluoride levels were directly proportional to bone porosity.

Deposition of fluoride also depends on the metabolic activity and vascularization of the particular bone or part of bone being considered. The effect of vascularization is immediate. Thus, the kinetics of disappearance of radioactive fluoride from the blood and its uptake by bone after a single intravenous injection have been used to measure bone blood flow. In one case the measuring was monitoring the positron emission (Van Dyke et al., 1965), and in another blood and urine activity were measured (Parker, 1972). The efficiency of removal of fluoride by the bone from blood on each pass is near 100%. The differential equations used by Parker (1972) to calculate the bone and urinary clearances of ^{18}F are shown in Figure 6.4. Bone blood flow can change in skeletal-associated disorders; for instance, Van Dyke et al. (1971) showed increased bone blood flow in myelofibrosis.

6.3.2.3 Fluoride in the Blood — Due to the relative low reabsorption and efficient uptake by bone, fluoride is cleared from blood rapidly. The fluoride blood concentration, following an oral dose of soluble fluoride, peaks in about an hour and then declines, so that in 4 to 5 hr the serum concentration is virtually normal (Hodge and Smith, 1970). When fluoride



$q_1(0)$ = INJECTED DOSE

$q_2(0) = q_3(0) = q_4(0) = 0$

$$\frac{dq_1}{dt} = k_{12}q_2 - (k_{21} + k_{31} + k_{41})q_1 \qquad (1)$$

$$\frac{dq_2}{dt} = k_{21}q_1 - k_{12}q_2 \qquad (2)$$

$$\frac{dq_3}{dt} = k_{31}q_1 \qquad (3)$$

$$\frac{dq_4}{dt} = k_{41}q_1 \qquad (4)$$

Figure 6.4. The compartment-system model and differential equations for calculation of bone and urinary ^{18}F clearance after a single intravenous injection. Source: Parker, 1972, Figure 1, p. 4.

is given intravenously, a rapid fall in concentration due to mixing is followed by a process with a half-time of 30 min (skeletal deposition) and then a process with a half-time of 2 to 3 hr (renal excretion). In general, fluoride occupies the same clearance space as chloride (i.e., equilibrates with the same extracellular fluid, but its distribution processes are slower). For instance, cells, including red cells, are easily permeable to fluoride (Underwood, 1971). However, passage across the cell membranes is slower for fluoride than for chloride. Thus in the experiments of Tosteson (1959), outflow rate constants for fluoride from cells labeled with radiofluorine were 0.3 per sec and for chloride, 3.1 per sec. Fluxes were 2,300 pmole/cm^2 per sec for ^{18}F and 13,100 pmole/cm^2 per sec for ^{38}Cl.

Hodge and Smith (1970) gave the typical total fluoride content of normal blood as 0.10 to 0.15 ppm. Singer and Armstrong (1960) found that in four communities in which the water contained 0.15 and 0.25 ppm fluoride, the mean plasma fluoride values of the residents lay within the range 0.14 to 0.19 ppm. In a fifth community, where the water contained 5.4 ppm fluoride, a slight increase to a mean value of 0.26 ppm was found. The data of Guy, Taves, and Brey (1976) indicated more clearly that the inorganic fluoride levels in plasma are directly related to the inorganic fluoride levels in drinking water. Plasma samples analyzed for fluoride ion concentrations from residents of Albany, New York (fluoride ion in drinking water less than 0.1 ppm), Corpus Christi, Texas (fluoride ion in drinking water of 0.9 ppm), and Andrews, Texas (fluoride ion in drinking water of 5.6 ppm), showed mean fluoride ion concentrations of 0.38 ± 0.21 *μM*, 1.0 ± 0.35 *μM*, and 4.3 ± 1.8 *μM* respectively.

Given the roughly equal distribution of fluoride between cells and plasma and the usual hematocrits, about 75% of the blood fluoride has been found in the plasma. The rest is mainly in or on red cells. Using dog blood, Carlson, Armstrong, and Singer (1960*b*) studied the changes in plasma content of chloride and fluoride induced by changes in whole blood carbon dioxide tension in vitro. A plasma fluoride shift qualitatively similar but quantitatively different than for chloride was found (i.e., at an elevated pH the increase in plasma chloride content was greater than for fluoride, and at a lower pH the decrease in chloride was less than for fluoride). Again, the lesser mobility of fluoride is seen.

Discrepancies between the fluoride content of plasma found by methods measuring total fluoride (Singer and Armstrong, 1960) and ionic fluoride (Taves, 1966) led to the realization that only a small part of fluoride in the plasma or serum, and possibly other body fluids, was exchangeable (i.e., in the form of free fluoride ion). Singer and Armstrong (1969) found free fluoride to be in the range of 0.004 to 0.008 ppm in the serum of individuals drinking nonfluoridated water, while in the serum of individuals drinking water fluoridated at a level of 1 ppm it was 0.01 to 0.02 ppm. Thus, exchangeable fluorine was about 20% of the total fluorine. Another indication of the ionic fluoride concentration is that about 15% to 20% of the total fluorine of normal human plasma is absorbed by calcium phosphate (Armstrong and Singer, 1970).

Taves and co-workers (Guy and Taves, 1972; Guy, Taves, and Brey, 1975; Taves, 1968) did extensive work on the nature of the nonexchangeable fluorine. They found that nonexchangeable fluorine in human plasma is mainly a derivative of perfluorinated octanoic acid and related substances. Taves, Grey, and Brey (1976) pointed out that perfluorinated octanoic acid derivatives are widely used commercially because of their surface-active properties. They suggested that the organic fluorine in human plasma is at least partly the result of contamination from industrial sources and probably has no relationship to fluoridation of drinking water. This view was confirmed by testing plasma from 106 persons living in five cities that had between 0.1 and 5.6 ppm fluoride in the drinking water. While the average inorganic fluoride concentrations ranged from 0.007 to 0.08 ppm and were directly related to the fluoride content of the water, the organic fluorine concentrations averaged 0.03 ± 0.016 ppm and showed no relationship to either the inorganic fluoride concentration or the total fluoride concentration of the water.

6.3.2.4 Fluoride in Soft Tissues — Soft tissues do not normally accumulate fluoride, and the low levels do not increase with age (Underwood, 1971, p. 377). Even in chronic fluorosis, increases in soft tissue concentrations of fluorine are small. Table 6.2 shows the fluoride levels in soft tissues of animals. The extreme case of supplementation of the feed of dairy cows

TABLE 6.2. FLUORIDE LEVELS IN SOFT TISSUES OF ANIMALS

Tissue	Fluoride level (ppm dry wt)					
	Rats		Sheep		Cows	
	Normal	2 mg fluoride per day for 76 days	Normal	10 ppm fluoride in water for 2 years	Normal	50 ppm fluoride in ration for 5.5 years
Liver	0.21	0.28	3.5	2.4	2.3	3.6
Kidney	0.62	1.50	4.2	20.0	3.5	19.3
Thyroid			3.0	7.6	2.1	7.3
Heart	2.6	5.4	3.0	2.3	2.3	4.6
Pancreas			2.8	3.2	2.8	4.2
Muscle	0.53	1.60				

Source: Adapted from Underwood, 1971, Table 44, p. 378. Data collected from several sources. Reprinted by permission of the publisher.

with 50 ppm fluoride for 5.5 years only increased the fluoride levels in heart, liver, thyroid, and pancreas two- to threefold above control levels; the high value for kidneys was probably due to retained urine. Following a temporary high intake of fluoride, the levels in the soft tissues quickly returned to normal. Carlson, Armstrong, and Singer (1960*a*) used radiofluoride to label 1 mg of fluoride given as a single dose to human volunteers. By monitoring fluoride concentrations with a probe-type scintillation counter, they found that the soft tissues lost nearly all their activity within 4 hr after ingestion.

Table 6.3 shows the concentration of fluoride in human soft tissues taken at autopsy following normal death, in relation to fluoride in the water supply. Table 6.4 is a condensation of the same data, giving the concentrations in parts of fluoride per million in the wet tissue rather than with respect to dry weight as given in the previous table. Table 6.5 shows the high levels of fluoride found in human tissues following fluoride poisoning fatalities and also shows the relatively small increase due to long- and short-term exposure to high atmospheric fluoride concentrations. It should be noted, however, that soft-tissue fluoride levels in fatally poisoned subjects depend on such parameters as dose and time of sampling after dose.

Some differences in the situation of fluorine in soft tissues may be discerned. For instance, while fluorine in the liver seems to be fully exchangeable with the ionic fluoride of the body fluids, about half the fluoride in the muscle is in a nonexchangeable form (Armstrong, 1967; Armstrong, Singer, and Vogel, 1966; Gedalia and Zipkin, 1973). There is also some indication of the existence in the brain of either a fluoride-passage barrier or a slow exchangeable fluoride pool (Armstrong and Singer,

TABLE 6.3. THE CONCENTRATION OF FLUORIDE IN HUMAN SOFT TISSUES IN RELATION TO THAT IN THE WATER SUPPLY

Age (years)	Sex	Fluoride in water (ppm)	Fluoride in desiccated tissues (ppm dry wt)					
			Heart	Liver	Lung	Kidney	Spleen	Aorta
65	M	0	2.12	2.48	1.79	2.50	1.83	106.1
62	M	0	2.44	3.26	4.21	2.59	3.23	23.4
50	M	0	2.49	2.79		2.79	3.63	4.39
47	F	0	1.39	1.45		2.90	4.24	4.18
32	M	0	2.47	1.68	4.06	2.83	2.84	2.64
40	F	0.5	1.22	0.92		1.35	2.17	10.0
32	M	0.5	4.29			4.19	7.31	2.65
85	M	1.0	2.00	3.49	10.5	3.97	7.19	47.98
83	F	1.0	2.53[a]	2.53	2.68	4.95	16.7	20.68
82	M	1.0	23.3	1.05	9.20	4.04	1.90	166.3
64	F	1.0	1.95	3.79	3.43	3.78	3.02	62.7
83	F	2.6	2.33	2.84	6.17	8.12	4.22	40.35
62	F	2.6	2.42	2.22	12.4	5.00	5.66	33.6
54	F	2.6	1.19	1.48	5.27	5.41	3.91	30.30
53	F	2.6	1.86	1.51	4.04	2.74	2.63	5.29
47	F	2.6	3.18	1.24		9.02	2.77	40.63
50	M	3.7	3.60	2.92	4.07	7.37	4.51	6.04
49	M	3.7	2.31	1.98				4.73
36	M	3.7	1.71	2.06		3.39		
36	M	3.7	1.84	1.01	3.31	2.28	1.34	3.09
71	M	4.0	6.81	1.96	11.17	16.8		46.01
53	M	4.0	2.65	2.59	4.70	7.63		59.4
26	M	4.0	3.46	5.41	4.46	25.6		6.21

[a] Heart aorta.

Source: Adapted from Smith et al., 1960, p. 330. Reprinted by permission of the publisher.

TABLE 6.4. CONCENTRATIONS OF FLUORIDE IN HUMAN SOFT TISSUES, NORMAL DEATHS

Fluoride in water supply[a] (ppm)	Fluoride in tissues (ppm wet wt)					
	Heart	Liver	Lung	Kidney	Spleen	Aorta
0.0 (7)	0.64 0.32-1.25	0.64 0.29-1.02	0.81	0.63 0.30-1.02	0.71 0.33-1.19	8.97 0.79-43.0
1.0 (4)	0.55 0.43-0.68	0.70 0.27-0.92	1.20 0.58-1.81	0.75 0.63-0.93	0.51 0.55-0.47	37.9 11.6-65.7
2.6 (5)	0.67 0.37-0.96	0.52 0.38-0.73	1.40 0.89-2.22	1.32 0.54-2.17	0.82 0.47-1.19	14.2 2.26-21.5
3.7 (4)	0.61 0.46-0.88	0.60 0.3-1.13	0.71 0.54-0.87	0.80 0.44-1.48	0.68 0.31-1.05	2.13 1.41-3.01
4.0 (3)	1.29 0.71-2.14	0.92 0.59-1.47	1.98 1.06-2.91	2.55 0.63-5.45		16.3 3.57-22.9

[a] Figures in parentheses indicate number of samples.

Source: Adapted from Hodge and Smith, 1965, Table IVB, p. 16. Reprinted by permission of the publisher.

TABLE 6.5. FLUORIDE CONTENT OF HUMAN TISSUES FOLLOWING FLUORIDE POISONING FATALITIES

Tissue	Fluoride (ppm fresh wt)			
	Normal[a]	Fluoride poisoning fatalities[a]	Exposure to high atmospheric fluoride concentration	
			Long-term	Short-term
Blood	0.27 (4)	9.2 (5)		
Brain	0.53 (4)	2.5 (2)	1.8	1.5
Lung	0.27 (3)	14.0 (2)	3.9	3.5
Heart	0.45 (3)	10.6 (1)	2.5	1.9
Spleen	0.28 (2)	11.8 (1)	1.7	1.8
Liver	0.54 (3)	9.3 (5)	1.6	1.4
Kidney	0.68 (2)	9.0 (3)	2.9	2.9
Thyroid			5.2	4.0
Aorta			29.4	28.2
Pancreas			1.8	1.7

[a] Figures in parentheses indicate the number of subjects.

Source: Adapted from Armstrong and Singer, 1970, Table 6, p. 101. Data collected from several sources. Reprinted by permission of the publisher.

1970). The concentrations tend to reflect those in the plasma (Carlson, Singer, and Armstrong, 1960). Some tissues such as the aorta, tendons, cartilage, and placenta, may have a high fluorine concentration because of fluoride fixed by ectopic calcification loci (Hodge and Smith, 1970).

Besides fixation on other calcified tissues, fluoride fixes secondarily on calcifications in the aorta (Waldbott, 1966). Some levels of fluoride intake may actually reduce calcification. Bernstein et al. (1966) reported on 300 subjects over age 45 living in an area (North Dakota) where the fluoride content of water was 4.0 to 5.8 ppm and 715 subjects where the fluoride content of the water was 0.15 to 0.3 ppm. They found the frequency of calcification of the aorta, as determined radiologically, to be significantly higher in the low-fluoride area, particularly in men; however, the reason was unexplainable. The results are shown in Figure 6.5. Studies with rat aorta in vitro (Zipkin et al., 1970), showing inhibition of calcium-45 uptake by incubated aorta (24-hr incubation) in the presence of fluoride, supported the above finding that fluoride in physiological doses may reduce calcification. When no fluoride was added, the calcium count per min per milligram of aorta was 1049 ± 21. After addition of 1.12 ppm fluoride, only 172 ± 4.1 calcium counts per min per milligram of aorta were measured.

6.3.2.4.1 Fluoride in the placenta — Fluoride passes from the mother to the fetus and is used in mineralization. Gedalia, Brzezinski, Zukerman, and Mayersdorf (1964) studied the placental transfer of fluoride to the human fetus at low and high fluoride intake. Samples of maternal blood, cord blood, and whole placental tissue were obtained at normal deliveries from 39 women in Israel where the water contained 0.5 to 0.6 ppm fluoride, from 12 women where the fluoride levels in water ranged from 0.6 to 0.9

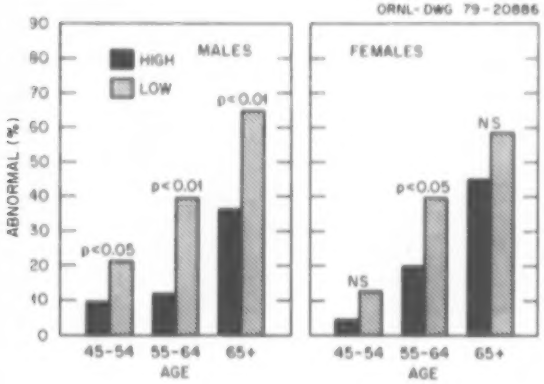


Figure 6.5. Percentage of radiologically detectable calcification of abdominal aorta in males and females residing in high- and low-fluoride areas. Source: Adapted from Bernstein et al., 1966, Figure 3, p. 501. Reprinted by permission of the publisher.

ppm, and from 18 women where water fluoride levels ranged from 0.5 to 0.6 ppm and who had also received a supplement of 0.5 mg of fluoride daily during the second half of pregnancy. Mean-total fluorine (not fluoride ion) concentrations for the first group and for groups two and three together, respectively, were: placenta, 0.121 and 0.228 ppm; cord blood, 0.165 and 0.175 ppm; and maternal blood, 0.150 and 0.234 ppm. These results were interpreted as showing that fluoride passes freely through the placenta when the fluoride intake is low (cord blood fluoride levels were slightly greater than maternal blood fluoride levels for group one women), but the placenta regulates fluoride transfer and protects the fetus when the intake is high (groups two and three cord blood fluoride levels were lower than placenta or maternal blood fluoride levels).

Ericsson and Malmnas (1962), however, in studies involving the maternal-fetal transfer of radioactive fluoride in humans and rabbits, showed that the protective function of the placenta may be only a passive one. Figure 6.6 shows these results. The bulk of the maternal fluid space of fluoride, quick clearance, and slowness of diffusion across the placenta offers protection to the fetus from unusual doses of fluoride. Ectopic calcifications in the placenta also offer protection by fixation of excess fluoride.

Using methods developed to distinguish between organic and inorganic fluoride discussed in Section 6.3.2.3.1, Shen and Taves (1974) studied the distribution of fluorine in the placenta, cord blood, and maternal blood. As shown in Figure 6.7, the fetal blood fluoride (16 deliveries) was parallel to, but lower than, the level in the maternal blood. Figures for the acid-labile (inorganic) fluoride and organically bound fluoride of the placentas are shown in Table 6.6. The organic fluoride levels are like those previously found in blood. The high levels of inorganic fluoride are postulated by the authors as being due to fixation by ectopic

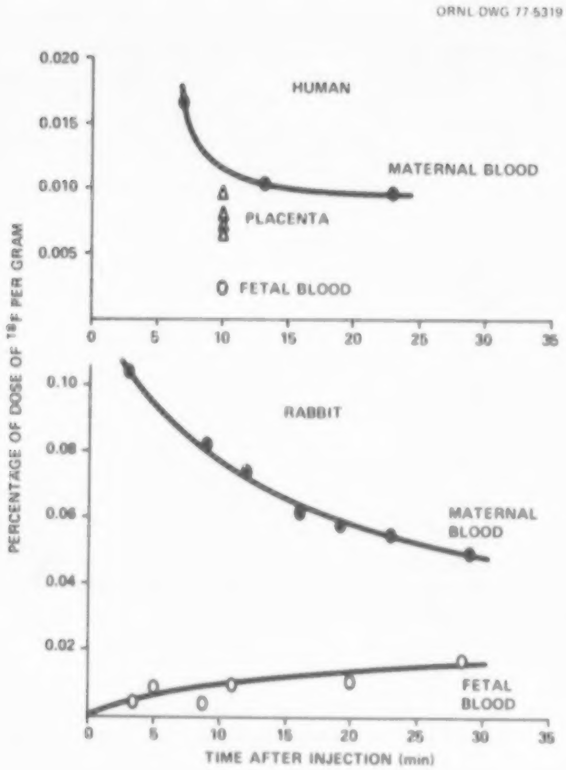


Figure 6.6. Maternal-fetal transfer of ^{18}F in the human and in the rabbit. Source: Adapted from Ericsson and Malmnas, 1962, Figure 1, p. 110. Reprinted by permission of the publisher.

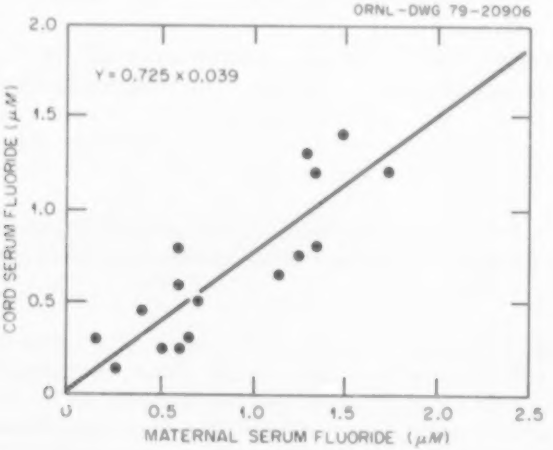


Figure 6.7. Scatter diagram of the maternal versus the cord serum fluoride values taken at the time of delivery. Source: Adapted from Shen and Taves, 1974, Figure 1, p. 206. Reprinted by permission of the publisher.

TABLE 6.6. FLUORIDE CONCENTRATION IN PLACENTA

Sample No.	Acid-labile fluoride (μM)		Organic fluoride (μM)	
	Electrode	Morinthorium reagent	Electrode	Morinthorium reagent
1	10.2	12.0	6.1	4.2
	40.6	33.5	3.5	7.5
2	80.2	101.2	8.1	7.0
	103.6	142.5	6.3	5.0
3	92.4	100.0	3.3	5.0
	67.4	53.0	4.1	2.8
4	19.6	14.0	3.0	5.6
	23.6	16.5		
5	6.6	14.8	1.9	1.5
	19.6	17.5	3.5	1.5
Mean	46.40	50.50	4.42	4.45
Std error	11.48	14.94	0.65	0.72

Source: Adapted from Shen and Taves, 1974, Table I, p. 206. Reprinted by permission of the publisher.

calcifications. Based on these data, the authors considered that it was not necessary to postulate a control mechanism for transfer of fluoride across the placenta, since the transfer could be explained by passive diffusion alone.

Auermann, Borris, and Grosser (1964) considered that the placenta served a secretory function protecting against cyclical changes in the blood of the fetus. The urine of women in labor and during pregnancy contains less fluoride than that of nonpregnant women.

Hanhijärvi, Kanto, and Ruponen (1974) studied the free-ionized plasma fluoride concentration of women during a normal pregnancy, a pregnancy complicated by toxemia, and during lactation. Their results are shown in Figure 6.8. Inorganic plasma fluoride, which decreased as pregnancy developed, returned to normal on lactation.

Some analyses for fluoride content of ashed fetal bones and teeth from areas of different fluoride water concentrations are shown in Table 6.7.

6.3.2.4.2 Fluoride in milk — Table 6.8 shows some fluoride values in human milk. Concentrations are generally less in human milk than in blood or drinking water. The level of fluoride in the milk of nursing mothers was studied by Simpson and Tuba (1968) in Edmonton, Canada, before and after fluoridation of the water supply. At the prefluoridation level of 0.26 ppm fluoride, the human milk concentration was 0.20 ppm; following fluoridation to 1.0 ppm, it averaged 0.49 ppm. For nursing infants this is about optimal. The authors considered that breast-fed infants needed

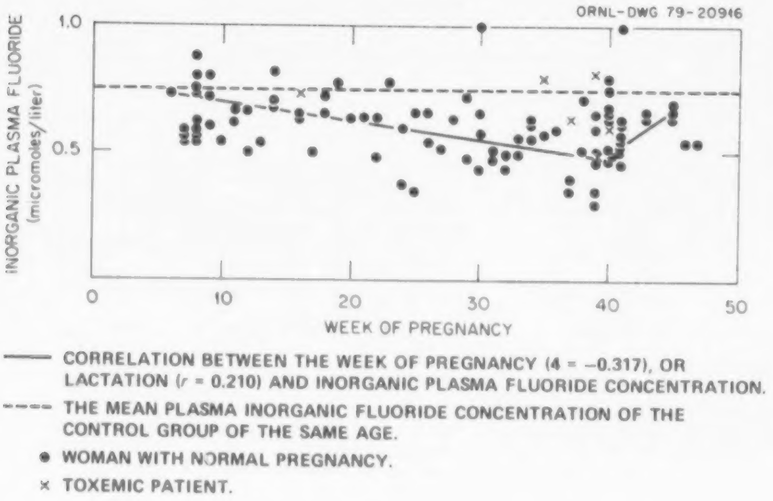


Figure 6.8. Inorganic plasma fluoride related to duration of pregnancy. Source: Adapted from Hanhijarvi, Kanto, and Ruponen, 1974, Figure 1, p. 144. Reprinted by permission of the publisher.

TABLE 6.7. FLUORIDE CONTENT OF ASHED FETAL BONES AND TEETH

Fetal age (months)	Number of cases	Mean fluoride value (ppm)		
		Femur	Mandible	Teeth
	Low fluoride area[a]			
6	21	39.7	42.3	30.9
7	24	40.7	39.0	34.0
8	13	42.3	38.5	31.7
9	27	43.8	46.9	40.8
	Medium fluoride area[b]			
6	31	59.0	47.0	32.6
7	20	71.6	53.5	43.0
8	7	79.4	66.0	57.9
9	34	92.5	78.8	69.7
	Elevated fluoride area[c]			
6	20	55.2	57.2	44.0
7	6	63.0	65.7	47.0
8	13	79.9	70.3	52.0
9	25	85.2	85.0	53.8

[a] About 0.1 ppm fluoride, Tel-Aviv, 1961-63.

[b] About 0.55 ppm fluoride, Jerusalem, 1961-63.

[c] About 1 ppm fluoride, Negev, southern Israel, 1961-64.

Source: Adapted from Gedalia et al., 1964, Table 3, p. 334, and Gedalia, Zukerman, and Leventhal, 1975, Table 2, p. 1122. Reprinted by permission of the publishers.

TABLE 6.8. CONCENTRATIONS OF FLUORIDE FOUND IN HUMAN MILK

Fluoride concentration (ppm)	Comment
0-0.35, av 0.131	4-5 days postdelivery; 0.55 ppm fluoride in drinking water
0-0.24, av 0.107	4-32 weeks postdelivery; 0.55 ppm fluoride in drinking water
0.08-0.147	Maternal blood contained 0.23-0.36 ppm fluoride
0.115-0.168	Mothers given 5 mg fluoride daily 2-6 days
0.10-0.18	Drinking water contained 0.055 ppm fluoride
0.25	Drinking water contained 1.13 ppm fluoride
0.13-0.25	Control subjects
0.45-0.60	Nonworkers living near superphosphate factory
1.05-1.88	Workers in superphosphate factory
0-12	Dry basis
Not detected to 0.12	3-8 days postdelivery; 0.06 ppm fluoride in water supply

Source: Adapted from Smith, 1966, Table 26, p. 104. Data collected from several sources. Reprinted by permission of the publisher.

no fluoride supplement where the community water supply was fluoridated. In communities where fluoride is low, the authors advised supplementation to bring the intake of the mothers to 1 mg/day. Contrasting results were presented by Backer Dirks et al. (1974). Milk samples were collected during the fourth and fifth days post partum from 11 mothers who lived in an area where fluoride concentrations in the drinking water were only 0.1 ppm and from 11 mothers who were living in an area where the drinking water had been fluoridated (1.0 ppm F^-) for the past 18 years. Their analysis of the milk samples, in contrast to the work of Simpson and Tuba (1968), indicated that the total fluorine values of the two groups of mothers were not significantly different.

6.3.2.5 Total Body Fluoride — Using data available for fluoride concentration in different human tissues, Smith (1966) calculated the amount and distribution of fluorine in man. The vital organs (kidneys, lungs, spleen, thyroid, heart, brain, pancreas, and liver) of a standard 70-kg man (International Commission on Radiological Protection, 1959) were assumed to weigh 5.04 kg, and fluoride therein was calculated to be 2.5 mg. Muscles and skin of a standard man comprise 30 kg and 2 kg, respectively, and the fluoride content was calculated to be 16 mg. For a blood weight of 5.4 kg and average whole-blood concentration of 0.11 ppm, the blood would contain 0.59 mg of fluoride, of which 0.48 mg would be in the plasma and 0.11 mg in the cells. The extracellular fluid is assumed to weigh 12.25 kg and to contain about 1.96 mg of fluoride. Bones and teeth account for 15.31 kg, and the fluoride content is assumed to be 2.55 g. Note that these fluoride calculations are derived from analyses of cadavers of persons who had used water containing about 0.1 ppm fluoride. The data are summarized in Table 6.9.

TABLE 6.9. CALCULATED DISTRIBUTION OF TOTAL BODY FLUORIDE IN HUMAN TISSUES

Tissue	Fluoride (g)	Percentage of total fluoride	Percentage of nonskeletal fluoride
Bones and teeth	2.547	99.16	
Skin and muscle	0.016	0.62	74.1
Vital organs	0.003	0.12	14.0
Whole blood	0.0006	0.02	3.0
Extracellular fluid	0.002	0.08	9.3
Total	2.5686	100.0	100.4

Source: Compiled from Smith, 1966, pp. 92-93.

6.4 EFFECTS

6.4.1 Effects on Enzymes and Cell Systems

6.4.1.1 Effects on Enzymes — In excess, fluoride is considered to be a poison, exerting its effects mainly through interference with cellular respiration by inhibition of key enzymes in the glycolytic cycle and electron transport. This is the first of the four major functional derangements distinguished by Hodge (1969) in fluoride poisoning. The other derangements are: shock caused by fluid and electrolyte loss due to changes in permeability, central vasomotor depression, and depression of activity of vascular smooth muscle. Fluoride has an effect in vitro on cholinesterase activity, but Hodge did not consider this important in fluoride poisoning. In fact, fluoride was shown (Albanus, Heilbronn, and Sundwall, 1965) to have an antidote effect in cases of cholinesterase poisoning by organophosphorus compounds. Renal changes occur in poisoning but are not the cause of acute death. Fluoride binds calcium as CaF_2, and the diminution in free-ionized calcium may cause pertubation of calcium-associated processes (e.g., blood-clotting, nerve action, parathyroid gland activity, and maintenance of cardiac regularity). Such toxicological effects are discussed in Section 6.4.3.2.

Some enzyme processes seem to be activated by fluoride at low physiological concentrations. Caution must be exercised in interpreting the results of tests done with cell systems, as inhibition of an enzyme in one pathway could make it appear that an enzyme of another pathway was being activated. Frajola (1959) stressed the usefulness of multiple simultaneous enzyme activity studies to elucidate such interactions. Polar graphing makes a dramatic display of the effects.

Paunio (1970) described the effect of fluoride (concentrations not given) on a number of highly purified enzymes in vitro. Among these were proteases, enzymes from dental plaque, dental pulp, gingiva, and phosphorylating enzymes from fetal mineralizing tissues. Some enzymes were inhibited; others were not affected; and in some, activation was observed at low fluoride concentration followed by inhibition at higher levels.

Fluoride combines with metals; for instance, calcium, cobalt, iron, magnesium, and manganese. It may inhibit enzymes by interfering with the metal when this is an essential part of an enzyme complex. An example of this is phosphoglucomutase. This enzyme catalyzes the reaction glucose-6 to glucose-1-phosphate. The reaction needs glucose-1,6-phosphate as a cofactor and magnesium. In the presence of fluoride, a magnesium fluoride complex with glucose-1-phosphate and the enzyme is formed, inhibiting the reaction (Najjar, 1948). Najjar observed that with a glucose-1-phosphate concentration of $2.2 \times 10^{-3} M$ and a magnesium concentration of $5 \times 10^{-4} M$, the concentration of fluoride that gave half maximum inhibition was $1 \times 10^{-3} M$.

Enolase is a key enzyme in glycolysis and is inhibited by fluoride. Warburg and Christian (1941, 1942) postulated that fluoride formed fluorophosphate, which combined with the activating magnesium of the enzyme to form an inactive complex. However, Peters, Shorthouse, and Murray (1964) found fluorophosphate per se to not be inhibitory, while fluoride and phosphate were. Evidently there is some concerted action of the fluoride and phosphate for inhibition. A similar situation prevails with respect to inhibition of succinic dehydrogenase by fluoride and phosphate together (Slater and Bonner, 1952). The presence of the fluoride and the phosphate enhance each other's affinity for the enzyme, resulting in inhibition. It may be noted that fluoride and arsenate also inhibit enolase (Warburg and Christian, 1942).

Fluoride and complexes of fluoride, such as HFF^-, may react with metals that are in prosthetic groups of enzymes and thus interfere with binding and processing of the normal ligands. Examples of this are found among the heme protein enzymes, for instance, catalase, peroxidase, and cytochrome oxidase. These reactions are relatively easy to reverse (Hewitt and Nicholas, 1963). Inhibition of cytochrome oxidase is important because it is terminal in the cytochrome chain, reducing oxygen to water.

Hewitt and Nicholas (1963, pp. 401-402) stated that it might be expected that many enzymes dependent on magnesium would be inhibited by fluoride (concentrations not given), especially in the presence of phosphate. The particular susceptibilities, however, vary greatly among different enzymes. Thus, while the polynucleotide phosphorylase enzymes that depend specifically on magnesium are insensitive to fluoride, the 5-nucleotidases are inhibited. Acid phosphatases are usually sensitive while alkaline phosphatases generally are not. Some pyrophosphatases are not inhibited, but erythrocyte pyrophosphatase was inhibited 50% by $2 \times 10^{-5} M$ fluoride ion (Naganna and Narayana menon, 1948).

Isoenzymes may be inhibited to different degrees by fluoride (Venkateswarlu, 1970, p. 181), perhaps due to differences in order of reaction and affinity and also to different activator requirements. Thus while human liver esterase is inhibited by fluoride, pancreatic and intestinal esterases are not. Magnesium-activated animal and plant glutamine synthetases are inhibited by fluoride more than the corresponding cobalt-activated ones.

Besides inhibiting enzymes by combining with a necessary metal, fluoride has been postulated (Venkateswarlu, 1970) to activate some enzymes by removal of metal inhibition.

Table 6.10 summarizes the effect of fluoride on some enzyme systems. Table 6.11 gives the responses of several enzymes to inorganic fluoride and some compounds giving rise to fluoride. Wiseman (1970) presented several tables documenting the effect of inorganic fluoride on enzymes. Many studies of fluoride inhibition have been made to delineate mechanisms of toxicity, to cause an intermediate to pile up in studies of dissimilation processes, or to show that fluoride at levels optimal for dental health is not deleterious to the organism. Concentrations producing noticeable effects are often many times those that would prevail in the body. For instance, a "50% inhibition" of cholinesterase at 0.1 *M* sodium fluoride and even a "detectable inhibition" at 0.001 *M* are well above the 10^{-5} *M* fluoride which is a higher limit (about 0.2 ppm) for fluoride in the blood.

6.4.1.2 Fluoride and Adenylate Cyclase — Even though a fluoride effect may be minor in itself, it may have some part in modulating a cell process. For instance, activation or inhibition of an enzyme may be part of control of permeability or of a transport process. Discussion of the effects of fluoride on broken cell preparations as noted experimentally, is given as an example of the type of involvement mentioned.

This system is important because adenosine 3',5'-cyclic phosphate (cyclic AMP) is the "second messenger" in a great number of hormone and other receptor-initiated actions. Cyclic AMP is formed from magnesium-ATP by the action of adenylate cyclase found in the plasma membrane of many types of cells. The receptor part of the enzyme is apparently on the outside of the cell and the catalytic part on the inside. The cyclic AMP, formed on stimulation of the enzyme, modulates actions within the cell, sometimes inhibiting but usually initiating a cascade of catalytic

TABLE 6.10. SUMMARY OF EFFECTS OF FLUORIDE ON ENZYME SYSTEMS

Enzyme	Concentration for effective inhibition (*M*)	Effect
Enolase	10^{-3}	Accumulation of phosphoglyceric acid
Acid phosphatase	10^{-2}	Increase blood organic phosphate? Lower inorganic phosphorus?
Alkaline phosphatase	10^{-2}	As above
Cytochrome *c*	10^{-3}	Cellular anoxia
Esterase	10^{-6}	Accumulation of lower fatty acids
Amylase	10^{-2}	Increase in salivary carbohydrates
Carboxylase	10^{-2}	Increase in pyruvate
Lipase	10^{-3}	Accumulation of higher fatty acids

Source: Adapted from Hodge and Smith, 1965, Table LIV, p. 183. Reprinted by permission of the publisher.

TABLE 6.11. EFFECTS OF INORGANIC FLUORIDE COMPOUNDS ON ENZYME SYSTEMS

Compound	Enzyme	Concentration	Effect
Ammonium fluorophosphate	Cholinesterase	0.01 *M*	50% inhibition
Fluoride	Pigeon liver cyclophorase	0.04 *M*	Reduced oxygen uptake in presence of malate by 50%
	Animal and plant glutamine synthetase	10^{-2} *M*	50% inhibition of cobalt-activated enzymes
		10^{-4} *M*	50% inhibition of magnesium-activated enzymes
	Yeast glucose dehydrogenase	0.01 *M*	30-40% inhibition
	Anserinase	10^{-3} *M*	10% inhibition
	Catalase	-log(F) = 2.3-3.3	50% inhibition
	Phosphorylase	0.02 *M*	6.4% inhibition
	Phosphatase, alkaline	Approx 0.5 *M*	50% inhibition
	Phosphomonoesterases of *Torulopsis utilis*	10^{-3}-10^{-1} *M* F	25-60% inhibition
	Acid phosphatase of human prostate	-log(F) = 2.0	About 98% inhibition in β-glycerol phosphate About 90% inhibition in yeast adenylic acid substrate About 65% inhibition in phenyl phosphate substrate
	Steroid sulfatase of *Patella vulgata*	0.005 *M*	9% inhibition
	Glucosulfatase of *P. vulgata*	0.005 *M*	79% inhibition
	Pyrophosphatase	2.5 × 10^{-5} *M* F	
Potassium fluoride	Phosphatase of *Penicillium chrysogenum*	50 μ*M* KF	Inhibited
	Phosphatase, alkaline	0.01 *M*	No appreciable effect
	Aspartase	0.1 *M*	Negligible inhibition
Sodium fluoride	Enzymes of rat liver, kidney homogenates	0.0001 *M* F	Inhibit activation of acetate without affecting pyruvate oxidation, oxidative rate, or level of adenine nucleotides
	Cytochrome system	Approx 0.001 *M*	50% inhibition
	Aconitase	0.01 *M*	No effect
	Citrogenase	0.01 *M*	Slight inhibition
	Esterases	0.003 *M*	Partial or complete inhibition
	Phosphatase, alkaline	0.01 *M*	No inhibition
	Phosphorylase	0.2 *M* NaF	Accelerates conversion of phosphorylase *a* to phosphorylase *b*
	Cholinesterase	0.1 *M* NaF	Almost complete inhibition
		0.01 *M* NaF	50% inhibition
		0.001 *M* NaF	Detectable inhibition
	Creatine phosphokinase	1 × 10^{-3} *M* NaF	21% inhibition of forward reaction
		6.5 × 10^{-2} *M* NaF	100% inhibition of forward reaction
	Alkaline phosphatase of adult and embryonic guinea pig cortex	0.02 *M* 0.002 *M*	No effect
	Muscle ATPase	10^{-3} *M*	Hydrolysis of ATP reduced to 87%; hydrolysis of inositine triphosphate reduced to 36% (normal 46%)
		10^{-2} *M*	Hydrolysis of ATP reduced to 38%; hydrolysis of inositine triphosphate reduced to 12%
	Penicillinase	0.2 *M*	Ineffective
	Glucuronidase	1% NaF	Complete inhibition of hydrolysis of dehydroepiandrosterone sulfuric ester
	Amylase	Up to 0.01 *M*	No effect
	α-Hydroxy acid oxidase, hog kidney	0.003 *M*	No inhibition
	Hexokinase	0.02 *M*	No effect
	Phosphomutase (rabbit muscle, heart, liver)	0.02 *M*	43-48% inactivation
	Phosphatase, acid	0.02-0.1 *M*	Inhibition
	Cholinesterase	0.5 mg/ml of beef serum .	Strong inhibition
	Acid phosphatases	10^{-3} *M* NaF	80% inhibition
	Cozymase	0.001 *M*	Inhibited
	Prostatic acid phosphatase		Protected against thermal denaturation

TABLE 6.11 (continued)

Compound	Enzyme	Concentration	Effect
	Pyrophosphatase	0.001 *M*	Inhibition >90%
	Potato acid, alkaline pyrophosphatases	10^{-3} *M* NaF	Inhibited
	Potato acid phosphatase	10^{-3} *M* NaF	Inhibited
	Phosphatase, acid (Basidiomycetes)	0.02 *M* 6.35×10^{-6} *M*	Nearly complete inhibition Inhibition
	Phosphatase, alkaline	0.01 *M*	Complete inhibition
	Intestinal alkaline phosphatase	0.01 *M* NaF	10% inhibition
	Sulfatase A of ox liver	0.01 *M* NaF	95% inhibition
	Enolase	0.125 m*M*	Inhibited fermentation, respiration yeast
	Phosphatase, acetyl	0.01 *M*	No inhibition
		0.05 *M*	50% inhibition
	Rat liver homogenate, kidney homogenate	0.01 *M*	Inhibited net breakdown of high-energy phosphate
	Homogenate of Flexner-Jobling tumor	0.01 *M*, 0.03 *M*	Increased respiratory rate
	Homogenate of human fibrosarcoma	0.01 *M*, 0.03 *M*	Increased respiratory rate
	Mitochondria of Flexner-Jobling tumor	0.01, 0.03 *M*	Increased respiratory rate
	Homogenate of mouse hepatoma 98/15	0.01 *M*	Inhibited respiratory rate
	Homogenate of mouse adrenal cortical tumor	0.01 *M*	Inhibited respiratory rate
	Homogenate of mouse Novikoff hepatoma	0.01 *M*	Inhibited respiratory rate
	Homogenate of mouse C3H mammary carcinoma	0.01 *M*	Increased respiratory rate
	Water-soluble enzymes of streptococci	42 ug NaF	Inhibited arsenolysis of citrulline
	Catechol oxidase	0.02 *M*	39% inhibition
		0.1 *M*	95% inhibition
	Succino dehydrogenase	0.02 *M*	37% inhibition
		0.1 *M*	50% inhibition
	Phosphatases of cell membrane of *Propionibacterium pentosacum*	0.023 *M* F	Phosphatases of cell membrane inhibited in organisms grown in 0.023 *M* F solution
	Carboxylase	2.4×10^{-3} *M*	63% inhibition
	Enolase	$0.5\text{–}1.67 \times 10^{-3}$ *M*	43-45% inhibition
	Esterase	10^{-3} *M*	Inhibition >85%
		10^{-4} *M*	7% inhibition
	Lipase	0.1 *M*	40% inhibition
	ATPase	1.5×10^{-3} *M* NaF	Depressed enhancing effect of pentachlorophenol
	Decarboxylase	1.3 mg	50% inhibition
	Cholinesterase	1:3000	Inhibited frog rectus muscle
	Decarboxylase of *Pseudomonas fluorescens*	0.01 and 0.001 *M* F	Inhibition
Sodium monofluorophosphate	Phosphorylase	0.0021 *M*	50% inhibition
	Alkaline phosphorylase	0.002 *M*	50% inhibition
Sodium, potassium, and ammonium fluorides, sodium fluorosilicate	Amylase	1.7-8550 ppm F in saliva	No effect
		0.76-760 ppm F in substract	No effect
Tantalum potassium fluoride	Succinic dehydrogenase	1×10^{-4} *M* TaK_2F_7	No significant effect

Source: Adapted from Hodge and Smith, 1965, Table LIII, p. 177-182. Data collected from several sources. Reprinted by permission of the publisher.

events that results in the observed hormone or other effect. The amplification may be considerable. The action of the cyclic AMP is stopped by rephosphorylation to adenosine triphosphatase (ATP) by a phosphodiesterase. The effect of fluoride on the system hindered the discovery of adenylate cyclase and cyclic AMP. In 1956, Sutherland and co-workers (as cited in Robison, Butcher, and Sutherland, 1971) studied the effect of glucagon and epinephrine on the action of liver phosphorylase on glycogen, by adding fluoride routinely to block the action of liver phosphorylase phosphatase. The fluoride was added to maintain the activity of any active glycogen phosphorylase formed. But fluoride stimulates adenylate cyclase, and it was not until fluoride was omitted from the mixtures that the hormone effects were noted. The fluoride effect on fat-cell ghosts parallels that of the hormone, as shown in Figure 6.9. Loss of activity muscle homogenates also follows a parallel course for the hormone and for fluoride, as shown in Figure 6.10.

The difference in effect of fluoride on intact cells and on cell homogenates, in this case of cells from adipose tissue, is shown in Table 6.12. In both experiments cited, the stimulation by fluoride in the broken cells was well above that due to the hormone. While fluoride has been found to strongly stimulate the activity of adenylate cyclase where it occurs in practically all broken cell systems studied, it has relatively little effect on intact cells and tissues. The reason for this is not known. The adenylate cyclase system is highly organized in its situation in the membrane. It may be that fluoride, in spite of the fact that it penetrates into cells (Section 6.3.2.3), does not locate where it can exert much action unless the cell's organization is disrupted. The compartmentation serves the function of keeping the cell's adenylate cyclase from being continuously stimulated by fluoride, as there is no obvious way for its action to be turned off, except possibly by interacting with metal ions such as calcium and magnesium in the cell.

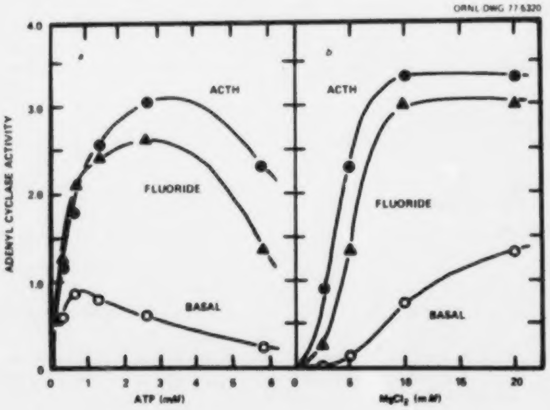


Figure 6.9. Effect of varying concentrations of ATP and Mg^{2+} on adenyl cyclase activity in fat cell ghosts: (*a*) activities are measured at fixed concentration of Mg^{++} (5 m*M*); (*b*) activities are measured at fixed concentration of ATP (5.84 m*M*). Source: Adapted from Birnbaumer et al., 1970, The Actions of Hormones on the Adenyl Cyclase System, Figure 4, p. 195. In: *Role of Cyclic AMP in Cell Function*, P. Greengard and E. Costa, eds. Raven Press, New York. Reprinted by permission of the publisher.

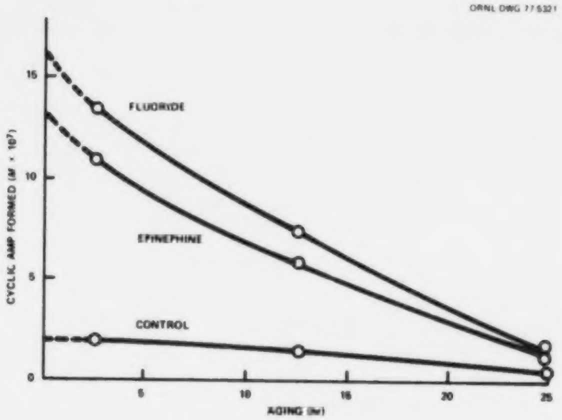


Figure 6.10. Loss of adenyl cyclase activity in a rabbit skeletal muscle homogenate at 4°C. Source: Adapted from Robison, Butcher, and Sutherland, 1971, Figure 2.4, p. 38. Reprinted by permission of the publisher.

TABLE 6.12. EFFECT OF EPINEPHRINE AND FLUORIDE ON CYCLIC AMP FORMATION BY ADIPOCYTES BEFORE AND AFTER HOMOGENIZATION

Experiment[a]	Conditions	Cyclic AMP (millimoles × 10^{-7}/g)	
		Whole cells	Broken cells
I	Control	2.4	7.8
	Epinephrine (1 μg/ml)	20.4	8.7
	NaF (0.01 *M*)	3.2	33.3
II	Control	3.8	13.0
	Epinephrine (1 μg/ml)	10.8	96.1
	NaF (0.01 *M*)	4.9	145.2

[a] Experiments I and II were performed at different times using different preparations of adipose tissue. For the experiments involving whole cells, adipocytes were prepared by the method of Rodbell and incubated in the absence of added ATP or Mg^{2+}. For the experiments with broken cells, the adipocytes were homogenized in an isotonic tris buffer (experiment I) or in a hypotonic glycylglycine-$MgSO_4$ buffer (experiment II) and incubated in the presence of 2 m*M* and 3 m*M* $MgSO_4$. Caffeine (1 m*M*) was present in all cases.

Source: Adapted from Butcher and Sutherland, 1971, Table 2.111, p. 40. Reprinted by permission of the publisher.

Fluoride has been used as a probe to bring out changes in the development of the adenylate cyclase system. An example is given in Figure 6.11, which shows the results of a study by Schmidt et al. (1970) on adenylate cyclase activity in particulate preparations from homogenized rat

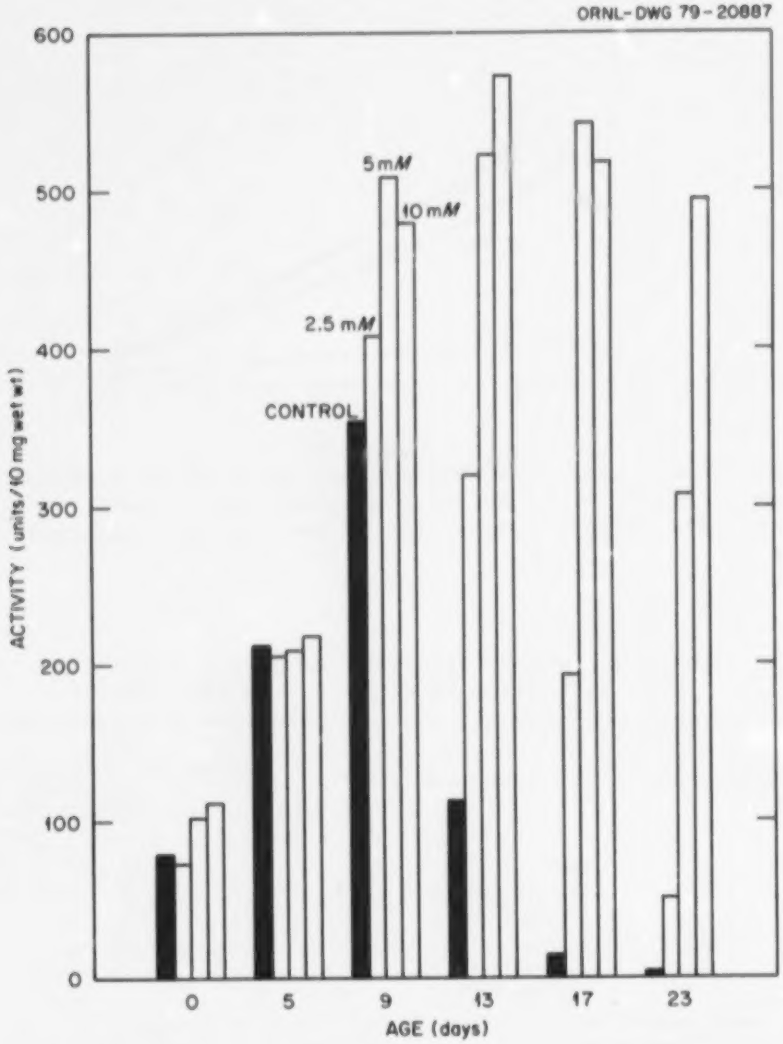


Figure 6.11. Effect of increasing concentrations of sodium fluoride on brain adenyl cyclase activity in rats. Source: Adapted from Schmidt et al., 1970, Figure 4, p. 59. Reprinted by permission of the publisher.

brain. Robison, Butcher, and Sutherland (1971) cited other studies showing the effect of developing at different times in different organs and varying among animals. They interpreted the results as showing that in the course of development, an inhibitory influence that can be reversed by fluoride (as 10 mM sodium fluoride) is superimposed on the adenylate cyclase system.

Menon, Giese, and Jaffe (1973) studied hormone- and fluoride-sensitive adenylate cyclases in human fetal tissues. The enzyme preparations from all tissues were stimulated by fluoride, but not all by the appropriate hormones. This indicated that some tissues develop their receptors or activate the mechanism sooner than others, leading the authors to postulate that cyclic AMP response may be a factor in control of developmental processes. A functional heterogeneity of human fetal anterior pituitary cells has been shown by Groom, Cooke, and Boyns (1971). While cyclic AMP stimulated release of both luteinizing hormone and follicle-stimulating hormone, fluoride augmented release mainly of follicle-stimulating hormone. The complexity of the adenylate cyclase system was shown by the differentiation of fluoride-stimulated and non-fluoride-stimulated components prepared from beef brain cortex enzyme in a study by MacDonald (1975), one component being activated by sodium fluoride and the other inhibited.

6.4.1.2.1 Fluoride and other cyclic nucleotidases — Another cyclic nucleotide, guanosine 5'-phosphoric acid (cyclic GMP) made from guanosine 5'-triphosphate (GTP) as cyclic AMP is made from ATP, has been found in all mammalian tissues studied (Hardman and Sutherland, 1969), usually in lower concentration than cyclic AMP. In contrast to its effect on adenylate cyclase, fluoride does not generally stimulate guanylate cyclase. Rodbell et al. (1971) found inhibition of fluoride stimulation of a glucagon-sensitive adenylate cyclase system from rat liver by guanyl nucleotides. Hosey and Tao (1975) found a similar inhibition of the system from rabbit red cells. These studies of broken cells are of interest because they indicate how factors in the cell affect the overall response, modulating the effect of the hormone on the outside. Yamashita and Field (1972) found that in intact thyroid cells (but not in liver), the levels of cyclic GMP and the incorporation of tritiated guanine into cyclic GMP increased in response to sodium fluoride. This suggests that in at least one intact tissue, fluoride activates a guanylate cyclase.

6.4.1.2.2 Mechanism of fluoride stimulation of adenylate cyclase — Roberecht et al. (1975), in studies of the stimulation of amylase secretion by either pancreozymin or fluoride in the perfused rat pancreas, noted an effect of fluoride anterior to a possible direct effect on the adenylate cyclase system. They postulated that while the hormone acts on the receptor, the fluoride acts by causing variations in the phosphorylation of membrane proteins. Aspects of possible mechanisms of stimulation by hormones and fluoride are given in the following schemes. Perkins and Moore (1971), studying activation of adenylate cyclase in membrane fragments of rat cerebral cortex, considered that fluoride acts by dissociating some part of the adenylate cyclase complex to relieve inhibition of the enzyme, this action being complementary to the action of the hormone.

Another activation scheme is given in Figure 6.12 (Najjar and Constantopoulos, 1973). Using as an analogy the action of fluoride on phosphoglucomutase, the authors postulated that fluoride or the hormone accepts a phosphate group directly from the cyclase (single-enzyme system) or from a phosphatase to activate the cyclase (multi-enzyme system). A criticism of this scheme is that fluoride and the hormone probably do not act at the same site; however, they must act very near to each other (perhaps on the same complex), as shown by the histochemical studies of heart and skeletal muscle stimulated by glucagon, epinephrine, and fluoride

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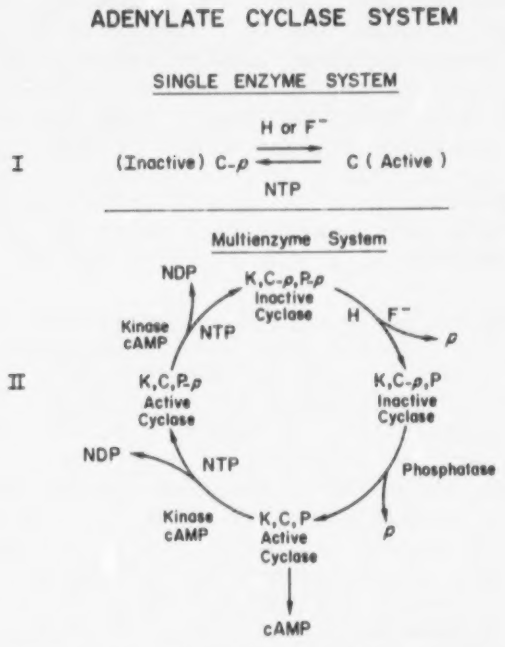
ADENYLATE CYCLASE SYSTEM
SINGLE ENZYME SYSTEM
I
H or F⁻
(Inactive) C-p
C (Active)
NTP
Multienzyme System
II
NDP
K,C-p,P-p
Inactive
Cyclase
H
F⁻
p
Kinase
cAMP
NTP
K,C,P-p
Active
Cyclase
K,C-p,P
Inactive
Cyclase
NDP
NTP
Phosphatase
Kinase
cAMP
K,C,P
Active
Cyclase
p
cAMP

H – HORMONE, F – FLUORIDE, NTP – NUCLEOSIDE TRIPHOSPHATE.

C – DEPHOSPHORYLATED ADENYLATE CYCLASE, C-p THE PHOSPHORYLATED FORM.

K – ADENYLATE CYCLASE KINASE WHICH MAY EXIST IN THE PHOSPHO AND DEPHOSPHO FORM, ACTIVE AND INHIBITED RESPECTIVELY.

P – DEPHOSPHORYLATED ADENYLATE CYCLASE PHOSPHATASE, P-p THE INHIBITED PHOSPHORYLATED FORM.

Figure 6.12. The proposed mechanism of activation of adenylate cyclase system. Source: Adapted from Najjar and Constantopoulos, 1973, Figure 1, p. 89. Reprinted by permission of the publisher.

(Schulze, Krause, and Wollenberger, 1972). The authors noted that of the known ATP-splitting enzymes, adenylate cyclase is the only one activated by fluoride; others are inhibited or not affected. It is interesting to note that along with a primary direct effect of fluoride on brain adenylate cyclase which increases production of cyclic AMP, Katz and Tenenhouse (1973) found an indirect effect of inhibition by fluoride of hydrolysis of ATP and ADP. Since these are inhibitory to phosphodiesterase, the secondary action of fluoride indirectly kept the phosphodiesterase from rephosphorylating the cyclic AMP to ATP, thus maintaining its level.

A highly formal model for adenylate cyclase action is shown in Figure 6.13. Discriminators (hormone receptors) on the surface of the membrane are postulated to act through transducer components to influence an amplifier molecule, which is the catalytic component of the complex. Fluoride is postulated to act on a site on the amplifier in such a way (the hormone also having this effect) as to enhance the affinity of a nonsubstrate site

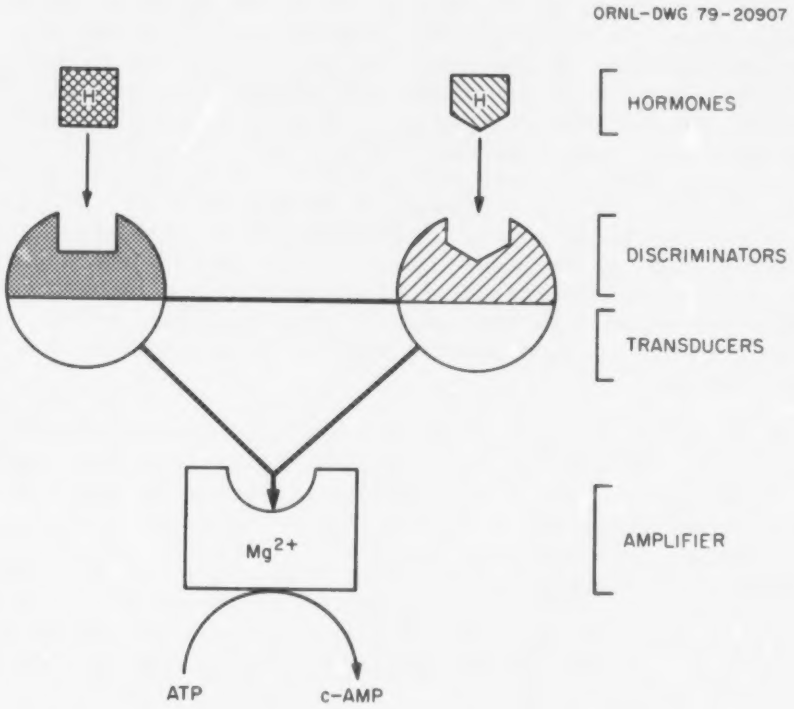

Figure 6.13. A schematic representation of the adenylate cyclase of the cyclase-PDE model cyclic AMP generating system. Source: Davies and Williams, 1975, Figure 2, p. 4. Reprinted by permission of the publisher.

for magnesium such that the magnesium binding enhances the activity of the substrate-binding site. While Severson, Drummond, and Sulakhe (1972) found that binding of magnesium enhanced reactivity, they did not find that the association constant, K_a, for this binding was altered by fluoride. They considered that fluoride, metal ions, and the hormone all act to increase the maximal velocity of the reaction by a "V" allosteric mechanism.

de Haën (1974) criticized the concept that there is a regulatory binding site for magnesium whereby the action of fluoride and hormones is affected. Magnesium ATP is seen to be the true substrate; ATP alone is inhibitory. It is postulated that hormones, as well as fluoride, decrease the sensitivity of the adenylate cyclase toward free ATP. Parallel to this, the maximal velocity is increased. Both effects are caused by an equilibrium conformational change in the enzyme. This scheme stresses steady-state operation of the system, with feedback from substrate binding and other factors influencing receptivity to the hormone.

6.4.1.3 Fluoride and the Thyroid Gland — Because both are halogens it was once thought that fluorine might be antagonistic to iodine and thus be goitrogenic. However, it seems that while chlorine and bromine are slightly concentrated by the thyroid gland, fluorine is not (Demole, 1970).

Fluoride, at a pharmacological dose of 50 to 100 mg daily, does have a mild antithyroid effect in hyperthyroid patients but not in normals. At one time fluoride was used in the treatment of exophthalmic goiter. However, the therapeutic action is inconstant and transitory, and such medication is obsolete. Fluorine-containing drugs synthesized for treatment of thyroid disorders have been a disappointment. The question of thyroid-fluoride effects has been reviewed by Demole (1970) whose conclusions were: (1) fluoride does not accumulate in the thyroid; (2) fluoride does not affect the uptake of iodine by thyroid tissue; (3) pathological changes in the thyroid show no increased frequency in regions where the water is fluoridated; (4) administration of fluoride does not interfere with iodine prophylaxis for endemic goiter; and (5) the beneficial effect of iodine in threshold dosage to experimental animals is not inhibited by even high doses of fluoride.

Differences in the action of fluoride, a thyroid stimulating hormone (TSH), and other stimulators were shown by Pastan, Macchia, and Katzen (1968). Thus while fluoride increased glucose-1 and glucose-6 oxidation, increased incorporation of phosphate into phospholipid, and stimulated adenylate cyclase in various broken cell preparations, it failed to induce the formation of pseudopods or intracellular droplets. Willems, Berberof-Van Sande, and Dumont (1972) similarly found an inhibitory effect of fluoride on accumulation of intracellular colloid droplets and secretion. They postulated that this was due to inhibition by fluoride of aerobic glycolysis in the follicular cells.

In studies with intact mouse thyroid glands, Williams and Wolf (1971) and Kendall-Taylor (1972) found that preincubation of the glands with fluoride (3 m*M* sodium fluoride) blocked the response to both TSH and cyclic AMP (adenosine-3,5'-monophosphate); iodoprotein was also released. These effects were considered to be due to metabolic toxicity of fluoride with secondary membrane-damaging effects; however, caution in interpretation was urged because of the multiple actions of the various agents (hormones, prostaglandins, and fluoride) and concentration effects.

McLaren (1976) reviewed the literature on the relationship of fluoride and the thyroid gland, with attention to reasons (e.g., physiological and experimental) for inconsistencies in the published data. Some retention or loading of the thyroid by fluoride is considered to occur. Fluorides at sufficient concentration are seen to produce morphological changes. However, the lower level of intake at which this may occur is open to discussion.

6.4.1.4 Fluorides and Cell Growth — Berry and Trillwood (1963) reported that fluoride at concentrations of 0.045 to 4.5 ppm in the culture media reduced the growth of HeLa cells and of strain L mouse fibroblasts. The report caused concern since these concentrations were described as being equivalent to those recommended for use in fluoridation of drinking water. Other authors, however, found different results. Albright (1964, 1966), using mouse leukemic lymphoblasts, found inhibition did not occur below 8 to 9 ppm. At 20 ppm, a growth inhibition of 29% in seven days was observed. This inhibition could be partially reversed by pyruvate but not by citrate. More than one mechanism of inhibition was indicated, one probably being the blocking of enolase. Armstrong et al. (1965), using HeLa cells to repeat and extend the work of Berry and Trillwood and also using esophageal cells, found no effect of fluoride on cell multiplication or on protein synthesis up to 10 ppm; at 15 ppm an incipient retardant effect on growth of the esophageal cells was noted. Albright (1964, 1966) and Armstrong et al. (1965) emphasized that the cell-culture results should be appraised in relation to the fluoride concentrations that really exist in the body fluids. With quick renal excretion and bone as a sink, plasma fluoride concentrations and concentrations in the soft tissues are well below the concentrations showing inhibitory effects in vitro.

The importance of intracellular-extracellular fluoride ratios is shown by the experiments of Suttie et al. (1974), who developed a fluoride-resistant strain of mouse fibroblast cells, able to grow in media containing 70 ppm fluoride. Although the parent cells at 50 ppm fluoride had an intracellular to extracelluar fluoride ratio of 0.3, the ratio of the resistant cells was 0.03.

6.4.1.5 Fluorides and Cell Permeability — Fluoride has an action on permeability of membranes. Part of this action is due to inhibition of enolase and other metabolic perturbations and part to a direct effect on the membrane itself, as shown by the study of Lepke and Passow (1967) with nonmetabolizing ghosts of human erythrocytes. Fluoride at a concentration of 40 m*M* (760 ppm) caused potassium loss from potassium-loaded ghosts into isotonic sodium chloride solutions at about the same rate as from similarly treated intact metabolizing cells. If this effect occurred, it would be obscured by metabolic effects at lower physiologic concentrations.

Feig, Shohet, and Nathan (1971), following Keitt's (1966) lead of using fluoride inhibition of enolase as a model system for study of inherited pyruvate kinase deficiency, found multiple effects of fluoride. Membrane ATPase was inhibited 40% at 1 m*M* (19 ppm) and completely inhibited at 5 m*M*; however, the incorporation of fatty acids into membrane phospholipids was unaffected until all the ATP was used. Fluoride was shown to react directly with red cell membranes in the presence of calcium, the combination inducing a massive cation leak. A higher concentration (10 m*M*) was needed for this effect than for the inhibition of enolase or ATPase. Addition of oxidant (methylene blue) regenerated NAD, which permitted some formation of ATP beyond the enolase block, prevented the massive cation and water loss (but not the normal cation leakage), and reestablished membrane phospholipid renewal.

Millman and Omachi (1972) found active sodium extrusion from red cells and NAD concentrations to decrease simultaneously on addition of graded levels of fluoride starting at 0.5 m*M*. Figure 6.14 shows the effects of 5 m*M* fluoride and 10 m*M* pyruvate on ^{22}Na release from red cells and on cellular NAD concentration. It is postulated that decrease in pyruvate due to fluoride inhibition of enolase causes a drop in the subsequent formation of NAD. NAD is utilized by the enzyme glyceraldehyde-3-phosphate dehydrogenase. As the level of NAD falls, so does the activity of the enzyme, followed by proportionate decreases in the rates of phosphoglycerate kinase and Na,K-ATPase.

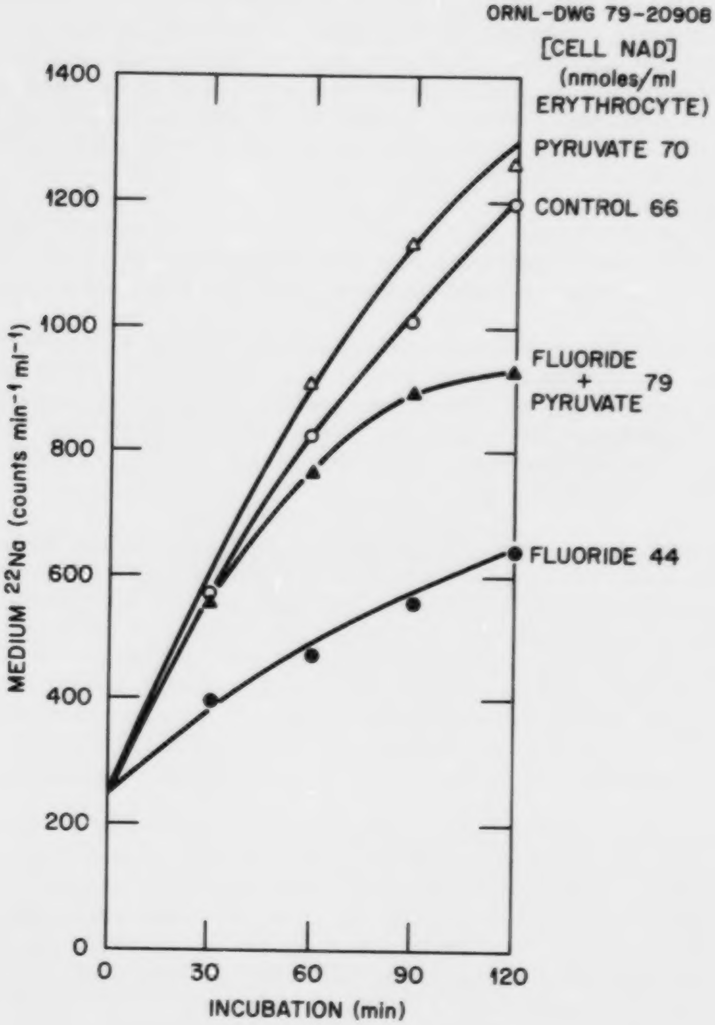

Figure 6.14. Effect of pyruvate on ^{22}Na release and cellular NAD concentration in fluoride-treated and control erythrocytes. Source: Adapted from Millman and Omachi, Figure 5, p. 344. Reprinted by permission of the publisher.

From the above studies, it is seen that at sufficiently high concentrations fluoride may be a factor, although, perhaps, only a minor one, in the dynamics of transport of electrolytes and fluids across cell membranes.

6.4.1.6 Fluoride and Protein Synthesis — Hardesty et al. (1973) showed that centrifugation of ribosome material from normal cells (reticulocytes) gave mostly aggregated material but gave a sharp peak of 78S material from ones incubated in fluoride. The sodium fluoride-treated ribosomes, if put into a cell-free protein-synthesizing system without fluoride, could incorporate *N*-terminal value and carry on with peptide elongation. However, regular ribosomes in the presence of sodium fluoride were inhibited. Thus fluoride appears to block a reaction of peptide initiation with concomitant accumulation of an initiation complex, explaining the resumption of chain elongation on removal of the fluoride.

Similar results were found by O'Rourke and Godchaux (1975). In a reticulocyte lysate system, some time was needed for polysome breakdown. Meanwhile, fluoride (at a level of 30 m*M*) allowed chain elongation but little initiation of new chains.

Marks et al. (1965) postulated that the derangement of the protein-synthesizing apparatus was secondary to inhibition of fluoride of ATP production, but this was not observed by Freudenberg, Halbreich, and Mager (1970).

The retention of *t*-RNA and messenger RNA on ribosomes following fluoride treatment (10 m*M*), postulated by Hardesty et al. (1973), is supported by the work of others (Baglioni, Jacobs-Iorena, and Meade, 1972; Geraghty et al., 1973; Terada et al., 1972). On removal of fluoride, polysomes reform and protein synthesis may resume. The dissociation of polysomes by fluoride has also been studied in cells other than reticulocytes — for instance, in HeLa cells (Baglioni, Jacobs-Iorena, and Meade, 1972) and mouse plasma tumor cells (Bleiberg, Zauderer, and Baglioni, 1972). In the latter study, addition of fluoride (15 m*M*) to mouse myeloma cells in culture caused disaggregation of polysomes and release of the fraction of ribosomes bound to the endoplasmic reticulum membrane. Removal of fluoride caused reaggregation and rebinding, and synthesis and secretion of immunoglobulin resumed.

Godchaux and Atwood (1976) studied the structure and function of the initiation complexes that accumulate during inhibition of protein synthesis by fluoride (20 to 30 m*M* potassium fluoride). Deacylated methionyl-*t*-RNA was found in the complex. The authors postulated that fluoride slows the use of the acylated RNA, allowing a deacylating enzyme to remove the methionine.

6.4.1.7 Fluoride in Inflammation, Wound Healing, and Bone Resorption — Stone and Willis (1968) studied interferences to inflammatory processes involved in the healing of skin disorders, skin bacterial infections, and the like, and found enhancement of inflammation by fluorides. Application of 0.5% (5000 ppm) fluoride by patch over skin scratches caused the formation of intradermal pustules. The authors felt that fluoride did not induce inflammation but enhanced it by exaggerating its early cellular phases. Joseph and Tydd (1973), studying regeneration of skin in the rabbit ear, considered that healing was stimulated by a wound hormone produced by cell damage and that fluoride might increase early cell damage and thus provide the stimulus. Increased acid phosphatase in the fluoride-treated tissues, indicating lysosomal breakdown, supported this hypothesis. Figure 6.15 shows the effect of fluoride at two concentration levels on the regeneration rates.

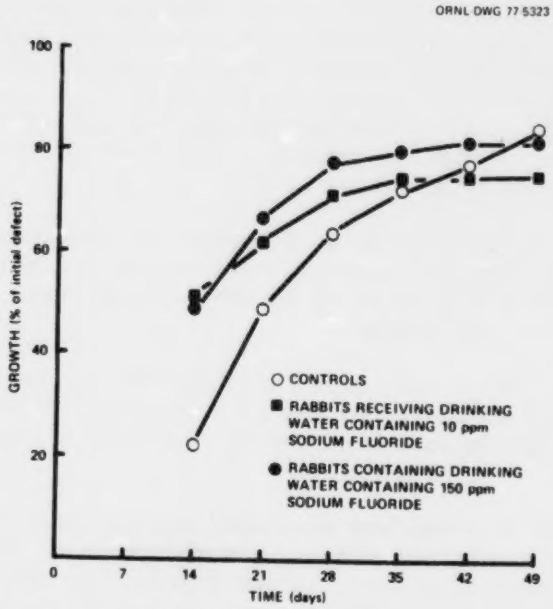

Figure 6.15. Effect of fluoride at two concentration levels on the regeneration of skin in the rabbit ear. Source: Adapted from Joseph and Tydd, 1973, Figure 1, p. 165. Reprinted by permission of the publisher.

Hars and Massler (1972) reported a beneficial effect of fluoride in dental surgery. Following a single injection of 1% stannous fluoride (0.3 ml) or 2% sodium fluoride (0.3 ml) into tooth extraction clots in rats, there was a lessening of bone resorption in the area around the socket. There were no noticed toxic effects on cellular morphology or bone structure, and healing of the epithelium and the subjacent tissue was maintained. The authors suggested the use of fluoride following extractions and tooth transplant and replant operations.

6.4.1.8 Functional and Cytochemical Effects — Manocha, Warner, and Olkowski (1975) maintained squirrel monkeys on distilled water and water containing 1 and 5 ppm fluoride for 18 months. Water consumption was higher at the higher fluoride intake. No changes in the nervous system were found. Slightly enhanced activity of citric acid cycle enzymes was noted in the liver of the higher-intake animals, and their kidneys showed some cytochemical changes. The activities of glomerular acid phosphatase, citric acid cycle, pentose shunt, and lysosomal enzymes were increased, indicating some degree of metabolic stress on the kidneys due to the higher fluoride regime and perhaps some catabolism. It should be noted that these symptoms resulted from a fluoride intake near the upper limit of the physiological range.

Löwe (1974) found some deterioration (flocky decay) of myofilaments of smooth muscle cells of guinea pig ileum. This deterioration was attributed to inhibition of glycolysis following incubation of the cells in 1 m*M* fluoride (19 ppm). This concentration is considerably higher than what these cells would ever encounter in vivo.

Czechowicz, Osada, and Slesak (1974) studied the effect of prolonged administration of sodium fluoride (daily intramuscular injection of 4 mg/kg for three months) on the metabolism of Purkinje's cells in the cerebellar cortex of guinea pigs. There was an intensification of the metabolic activity of the mitochondria and activation of membrane transport enzymes. The authors postulated that the changes reflected excitation of neurons by the added fluoride.

Fluoride (0.1 to 10 m*M* sodium fluoride) was shown to have an anticurare effect (increasing as the fluoride concentration increased) (Koketsu and Gerard, 1956), sensitizing postsynaptic receptors to acetycholine following depression of end-plate potentials by the curare (5×10^{-6} tubocurarine). Jacobs and Blaber (1971) studied this action at the neuromuscular junction of cat tenuissimus muscle. Transmitter store was increased from a control value of 1042.96 ± 175.75 to 1465.19 ± 150.45, and transmitter release was increased from a control value of 0.2261 ± 0.0310 to 0.2318 ± 0.0241 by 8 m*M* fluoride over the 3 μg tubocurarine block. Part of the fluoride effect was considered to be displacement of the drug from presynaptic sites. Fluoride acts as a noncompetitive, reversible inhibitor of erythrocyte cholinesterase (Heilbronn, 1965*a*). It also has been shown to reactivate cholinesterase blocked by organophosphorus inhibitors at concentrations of 0.5 to 5×10^{-3} *M* sodium fluoride (Heilbronn, 1965*b*) and 2 and 4 m*M* sodium fluoride (Blaber, 1970). Thus fluoride seems to have both pre- and postsynaptic effects.

Fluoride is one of the positive inotropic agents affecting in vitro heart muscle contraction (Robison, Butcher, and Sutherland, 1971). The response is seen at slow frequencies of contraction and at fluoride concentrations of 0.11 to 4.3 m*M* (Berman, 1966) and is reflected as a prolongation of the active state of contraction (Reiter, 1965).

6.4.2 Fluoride and Teeth

6.4.2.1 Fluoride, Fluorosis, and Tooth Health — Fluoride is utilized in the calcification of teeth in utero (Section 6.3.2.4.1). It is also present in the enamel before teeth erupt. Maier (1972) detailed how fluoride was found to have an effect on teeth health. Eager (1901), an American physician in Naples, Italy, upon examining prospective immigrants to the United States, reported the severe tooth mottling observed in persons from a small nearby community. He postulated that volcanic emanations were the cause, either in the air or forming a solution in the water. Cases from communities elsewhere in the world were then reported. In 1925 and 1928 the communities of Oakley, Idaho, and Bauxite, Arkansas, respectively, where mottling was a problem, changed their water supplies on the basis of a recommendation by dentist Dr. F. S. McKay. Dr. McKay thought that something in the water supply was responsible for the mottling, and after the water supplies were changed, no new cases appeared.

However, fluoride was not yet implicated. A report by Kempf and McKay (1930) was read by officials of the Aluminum Company of America (ALCOA). These ALCOA officials were concerned that the dental defects in persons living in Bauxite might be due to ingestion of aluminum. Accordingly, water samples from Bauxite were sent to ALCOA's laboratory in Pittsburgh where they were analyzed under the direction of the laboratory's chief chemist, Dr. H. V. Churchill. Analysis of the water gave 14 ppm fluoride. Water samples from other communities where tooth mottling was a problem were also analyzed by the company, and high fluoride levels were found. Fluoride was, therefore, indicated as the cause. The next step was to confirm these findings in animal tests. This, however, had already been done by Smith and co-workers in Arizona. Smith et al. (1931) produced tooth mottling in rats by feeding them fluoride. In addition to the analysis work of Churchill and the research of Smith et al. (1931), Velu (1931) in North Africa also concluded that fluoride was involved in tooth mottling. By giving sheep in North Africa water saturated with a fluoride-bearing rock phosphate, Velu (1932) produced a tooth mottling similar to that affecting many Moroccans. Meanwhile, the low incidence of caries in mottled teeth was noted, and appreciation of the balance between the beneficial effect of fluoride at low concentrations and deleterious effects at higher concentrations was obtained. During the decade 1937 to 1947, Dean did extensive work on chronic endemic dental fluorosis using rigorous epidemiological standards. Using the data of Dean, Arnold, and Elvove (1942), Hodge (1950) showed that the decrease in dental caries was a linear function of the logarithm of the fluoride concentration. The increase in fluorosis index is also log-linear, showing both to be true pharmacologic responses. This is shown in Figure 6.16, which includes the results of Dean, Arnold, and Elvove (1942) and Striffler (1958). Water intake changes with temperature and a graph of the relationships among fluoride levels, fluorosis, and ambient temperatures are shown in Figure 6.17.

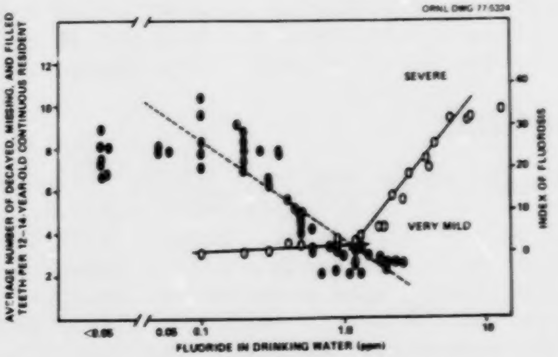

Figure 6.16. Relation between decayed, missing, and filled teeth (broken line at left), severity of fluorosis (solid lines), and fluoride concentration in water (logarithmic scale). Source: Adapted from Hodge and Smith, 1965, Figure 35, p. 465. Data collected from several sources. Reprinted by permission of the publisher.

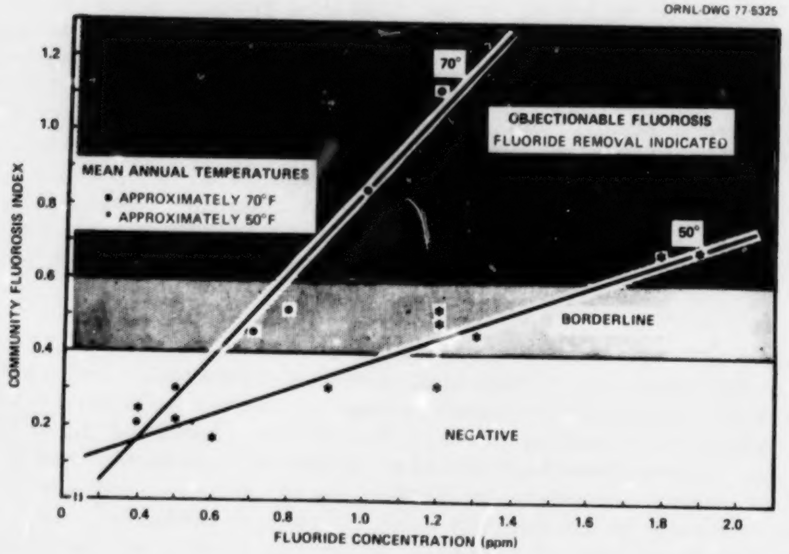

Figure 6.17. Relationship among fluoride levels, fluorosis, and ambient temperatures. Source: Adapted from Maier, 1972, Figure 13, p. 32. Data collected from several sources. Reprinted by permission of the publisher.

Galagan and Vermillion (1957) derived a formula for fluoride: parts per million of fluoride = $0.34/E$, where E is the estimated average daily water consumption of children through ten years of age in terms of ounces of water per pound of body weight. It may be obtained from the equation $E = 0.038 + 0.0062 \times$ average maximum temperature in °F. This formula is used in making up the U.S. Public Health Service Drinking Water Standards.

Excess fluoride results in dental mottling. The mottling occurs when enamel is being laid down; beyond 12 to 14 years teeth can no longer be mottled, whatever the fluoride intake. Very mild fluorosis is present in about one resident in five at a fluoride drinking water concentration of 1 ppm (Figure 6.16). In its mild form caused by exposure to 2 ppm fluoride in drinking water, fluorosis is manifested as chalky white flecks on the teeth; more severe forms of fluorosis (from exposure to 4 ppm fluoride in drinking water) may cause the flecks to show a brown discoloration, which is no doubt secondary to the actual histological damage. McKay and Black (as cited in Bhussry, 1970) showed a relative lack of interprismatic substance in the mottled areas, and Williams in 1923 (as cited in Bhussry, 1970) showed easier penetration of dyes into the mottled areas than into sound enamel. Later studies using microradiography, polarized light, and other techniques confirmed the histologic damage, showing irregular hypomineralization in the mottled areas.

The effect of fluoride in the mottling process seems to be mainly on the ameloblasts. In experimental animals the presence of excess fluoride caused thickening of the cell walls, elongation of the nucleic, and disruption of the internal cell organization (Fleming, 1953). Kruger (1970*b*) showed distension of the endoplasmic reticulum and enlargement of mitochondria in ameloblasts from young rats; the effects were proportional to time after injection and dose of fluoride (0.1, 3.0, and 7.0 mg of fluoride per kg body weight). Episodes of fluoride administration can be followed in the teeth by resulting paired hyper- and hypomineralized zones, followed by normal mineralization as shown by Walton and Eisenmann (1974).

Kruger (1970*a*) showed a reduction in the uptake of proline by ameloblasts in the presence of mottling doses of fluoride. In a later study, Kruger (1972) observed increased uptake of serine at submottling doses and reduced uptake at mottling doses. Proline is important in collagen and also in the interprismatic fibrillar material. Glimcher and Krane (1964) identified serine phosphate in bovine enamel matrix, and its presence there was considered to satisfy some of the criteria for crystal growth. The stimulating effect of fluoride on serine uptake at the lower, submottling doses supported the general observation of Hodge and Smith (1968) that "the primary effect of small doses of fluoride is the stimulation of the osteocyte (or the ameloblast). Larger doses impair the function of the osteocytes and the ameloblasts."

Dean's conclusions on fluoride in the water and dental health follow (as cited in Maier, 1972). The conclusions are the following: (1) When the fluoride level exceeds about 1.5 ppm, any further increase does not significantly decrease the decayed, missing, and filled (DMF) incidence but does increase the incidence and severity of fluorosis. (2) In a temperate climate at a level of about 1.0 ppm, optimal effects are observed — maximal reduction in caries with no esthetically significant mottling. Among 12- to 14-year-old children, DMF rates in permanent teeth were reduced by about 60%. (3) Fluorosis can be produced only during the period of calcification of the teeth (i.e., up to about 12 years), not thereafter. (4) Once lesions of fluorosis are formed, they cannot be repaired (as by medicinal or dietary influences) either during the calcification period or thereafter. (5) Fluorides appear to be the only agents ordinarily a part of the diet that have an influence on enamel formation. (6) After calcification is completed, the structure of the enamel remains unaltered despite subsequent changes in the diet.

Although fluorides may be the only agents ordinarily a part of the diet that have a direct influence on enamel formation, some substances (e.g., trace elements) may interact with the fluorides and have an indirect effect on enamel formation. Hadjimarkos (1967) found that simultaneous administration of selenium and fluoride reduced the beneficial effect of fluoride, while Stookey and Muhler (1964) showed that addition of molybdenum enhanced it. Bibby and Little (1975) showed incorporation of titanium, along with fluoride, in the organic part of the enamel and gave special attention to the role of organic fluoride in enamel.

6.4.2.2 Physiological and Biochemical Aspects of Caries Resistance and of Fluorosis — Research on the penetration of fluoride and associated cations into the tooth surface led to the advent of fluoridated toothpaste. Hoerman et al. (1966), using electron-probe microanalysis, found that fluoride applied in a stannous compound reached a level in the first few microns of enamel of about 500 ppm, which represents about a 20% increase over normal by observed levels. Slight acidification has been shown to increase fluoride uptake. Friberger (1975) considered that two factors largely explain this: (1) faster diffusion of hydrogen fluoride than of F^-, hindered by a hydration shell as well as by reaction with counterions, and (2) faster recrystallization at lower pH.

Jongebloed, Molenaar, and Arends (1975), by scanning electron microscopy, studied sections of extracted teeth subjected to artificial caries attack and treatment with sodium monofluorophosphate, one of the agents used in fluoridated toothpastes. The microscopy showed plugging of the interprismatic channels by material derived from the monofluorophosphate. The authors postulated that this clogging not only slowed the inroads of caries but also hindered removal of dissolved material, allowing redeposition when the caries attack subsided.

Levine (1976) reviewed the action of fluoride in caries prevention. In spite of much research, the mechanisms of this therapy are not fully understood. One possibility is reduction of enamel solubility by formation of fluorapatite. Increased crystallinity and increased precipitation of mineral in the presence of fluoride may also be factors, as well as the plugging mentioned above. Perdok (1962) presented evidence for a greater degree of hydrogen bonding between enamel protein and mineral material for fluorapatite compared with hydroxyapatite, resulting in a lower lattice-free energy and enhanced resistance to acid attack. Fluoride absorbed during development affects tooth morphology, causing rounder tooth cusps and shallower fissures. However, the effect of this morphological alteration on caries resistance is considered minor. Fluoride appears in dental plaque and has a bacteriostatic effect. Not only is bacterial glycolysis inhibited but also synthesis of polysaccharides which serve as a reserve for acid production and as a matrix which would hold fermentation products against the enamel surface. Birkeland and Charlton (1976), studying plaque removed from subjects in a 1 ppm fluoride area, showed an increase of fluoride ion activity upon fall in pH, with a resultant caries-resistant effect.

Although fluoride is important in enamel, the concentration there is not as great as in other hard tissues. The content is highest in the surface enamel. Thus, Weatherell and Hargreaves (1965), removing layers of enamel from an incisor of a 38-year-old male resident in a district where fluoride in the water was 0.5 ppm, found that fluoride concentrations fell steeply at first and then leveled off from 500 ppm at the surface to 50 ppm at about two-thirds enamel depth. Fluoride concentrations in tooth parts are shown in Figure 6.18 (Yoon et al., 1960). The high concentration in the pulp chamber should be noted, reflecting a good systemic supply of fluoride. The cementum, structurally related to bone, had a fluoride concentration of 2390 ppm. Singer and Armstrong (1962)

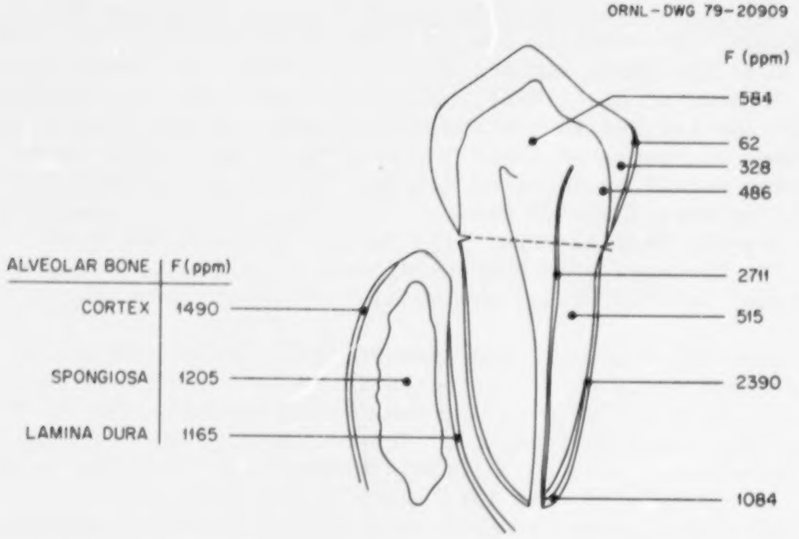

Figure 6.18. Fluoride concentrations in portions of alveolar bone and teeth. Source: Yoon et al., 1960, p. 569. Reprinted by permission of the publisher.

and Weatherell (1966), on the basis of postmortem studies showing good correlation between the fluoride content of the cementum (higher than bone) and that of the skeleton, suggested analysis of cementum biopsy samples as a way of estimating skeletal fluoride burdens. The patients' histories would have to be considered when doing this, as shown by the results of a detailed study by Stepnick, Nakata, and Zipkin (1975) of the effects of age and fluoride exposure on the fluoride, citrate, and carbonate content of human cementum. External fluoride exposure, disease, and abrasion were factors obscuring the relation.

Fluoride content of the teeth increases during life, but the rate of increase slows with age. Figure 6.19 (part *a*) shows the results at different concentrations of fluoride in the drinking water for enamel, and part *b* shows the results for dentin (Jackson and Weidmann, 1959). The greater incorporation and the less pronounced plateau in the dentin reflects the greater accessibility of this part of the tooth than the enamel to systemic fluoride.

The importance of early intake of fluoride for caries prevention is shown by the results of a study of 1500 children from infancy to 10 years of age (Margolis et al., 1975). Diminution of caries, ranked from the greatest retardation to the least, is listed: the group in a nonfluoridated community that had received a fluoride-vitamin supplement from infancy, the group living in an area where water was fluoridated, the group given fluoride-vitamin supplementation starting at age 4, and the control group. These results indicate the importance of fluoride during the infant years (0-4) for the reduction of dental caries.

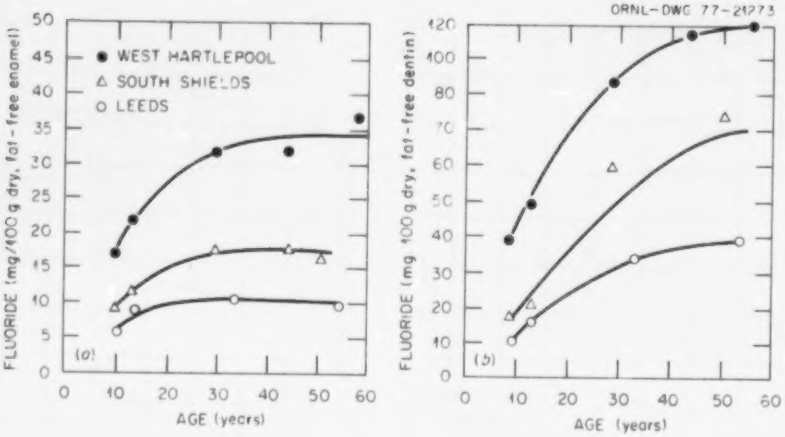

Figure 6.19. The relationship between age and the fluoride content of human premolar (*a*) enamel and (*b*) dentin. Source: Adapted from Jackson and Weldmann, 1959, Figures 1 and 2, p. 304. Reprinted by permission of the publisher.

6.4.2.3 Control of Fluoride as a Public Health Measure — On the basis of the epidemiological studies of tooth mottling and tooth decay discussed in Section 6.4.2.1, adding fluoride to public water supplies in which it was deficient was suggested by a number of authorities in the late 1930s and early 1940s. The suggestion was actually implemented in 1945 in Grand Rapids, Michigan, Newburgh, New York, and Brantford, Ontario. The U.S. Public Health Service (as cited in Maier, 1972) reported that in 1971 over 86 million persons in the United States were using fluoridated water, plus an estimated 9.5 million using public water that naturally contained sufficient fluorides. This, at the time, was about 58% of the people in the United States who were served by a public water supply. Figure 6.20 shows the growth in fluoridation from 1960 to 1970. It was reported in 1975 (Environmental Health Resource Center, 1975) that in the United States nearly 100 million persons used optimally fluoridated water, 115 million did not have its benefits, and some 150 million people in 30 countries around the world were drinking fluoridated water.

The benefits of fluoridation as a public health measure are beyond question. Cox (as cited in Hodge and Smith, 1965) evaluated data from cities where fluoridation had been introduced and described fluoridation as the most practical means of approaching the goal of sound teeth for all children. The dental benefits of fluoride in drinking water also extend to adults who continue to reside in communities with optimal fluoridation.

Figure 6.16, relating the fluorosis index, dental caries, and fluoride content of the drinking water, is shown in Section 6.4.2.1. Similar data are presented in a different way in Figures 6.21 and 6.22. The threshold for beneficial results is considered to be 0.5 ppm fluoride.

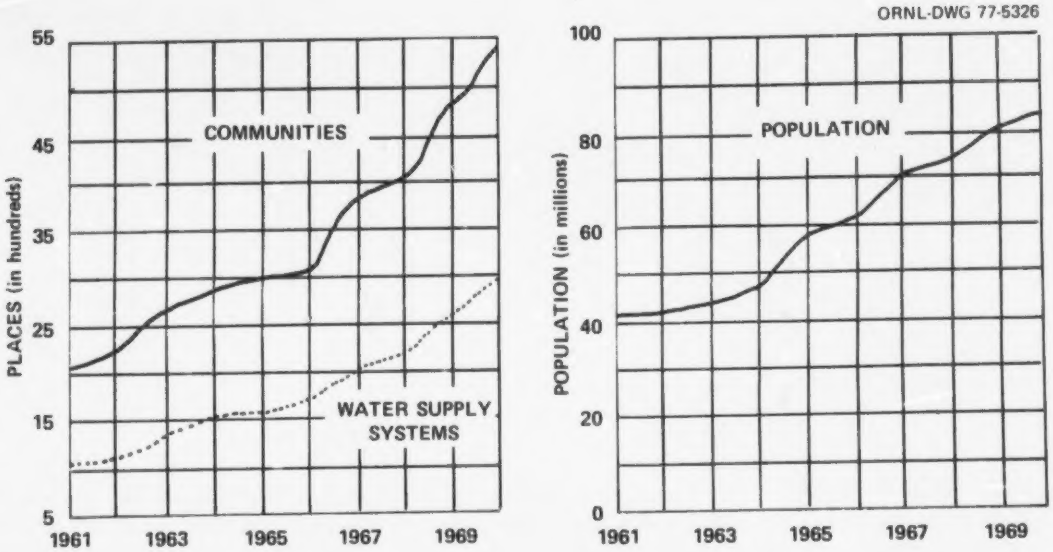

Figure 6.20. Communities, water-supply systems, and population served with fluoridated water, 1960-1970. Source: Adapted from Maier, 1972, Figure 10, p. 26. Reprinted by permission of the publisher.

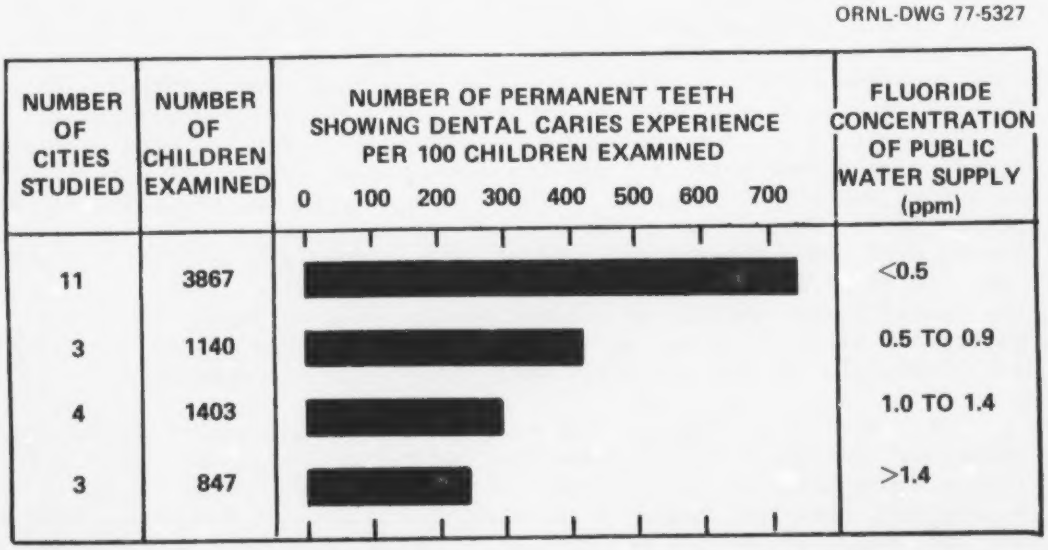

Figure 6.21. Relationship between fluoride concentration of public water supply and number of dental caries in permanent teeth of children. Source: Adapted from Maier, 1972, Figure 4, p. 18. Reprinted by permission of the publisher.

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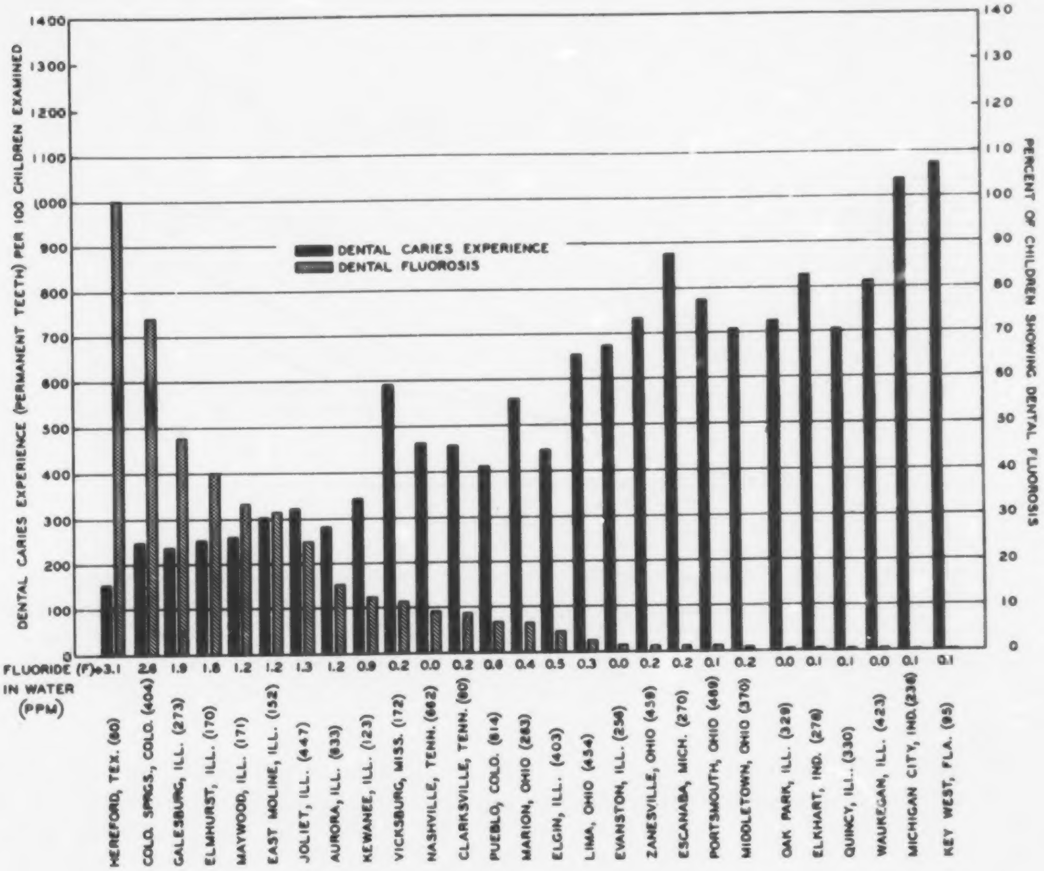

Figure 6.22. Relationship between dental caries prevalence (permanent teeth) and fluoride ingestion as measured by percentage of children showing dental fluorosis in 8576 selected 12- to 14-year-old children in 27 cities. Source: Adapted from Maier, 1972, Figure 3, p. 17. Reprinted by permission of the publisher.

The trade-off between fluorosis and caries prevention is clearly seen in Figure 6.22; the optimum fluoridation level in a temperature climate is considered to be about 1.0 ppm.

The long-term result of fluoridation in Grand Rapids, Michigan, is shown in Figure 6.23. The fluoride content of the water was 0.15 ppm before fluoridation and was raised to 1.0 ppm. After 15 years the curve of DMF teeth for Grand Rapids was approaching that of Aurora, Illinois, where the water is naturally fluoridated at 1.2 ppm.

The reduction of caries incidence from the Grand Rapids' baseline before fluoridation is very notable and is similar to the reduction in

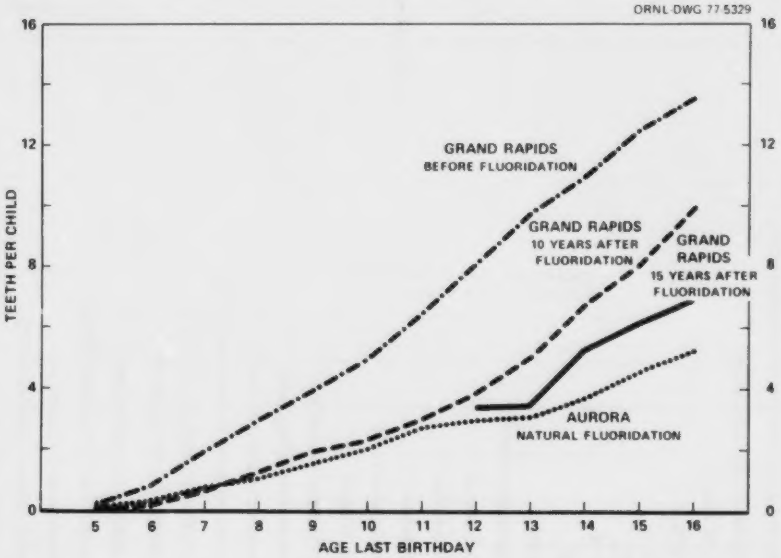

Figure 6.23. Reduction in caries incidence in permanent teeth of children from Grand Rapids, Michigan, after 10 and 15 years of fluoridation. Source: Adapted from Maier, 1972, Figure 6, p. 20. Reprinted by permission of the publisher.

all other communities studied. Fluoride is mainly effective in reducing the number of caries when taken during the period of tooth formation. As seen in Section 6.2, it is also useful for offsetting osteoporosis in middle-aged and elderly people. Because of the slow turnover of enamel material, the tooth effect largely lasts through life.

6.4.2.3.1 Possible countraindications to fluoridation — From time to time, various ills (e.g., acne, allergies, headaches, and numbness) have been attributed to fluoride in the water. Aside from a few well-documented cases of fluoride contact dermatitis under special circumstances (Smith and Hodge, 1959), the purported cases turn out to have no basis in fact. Perkins (1952) claimed that cancer deaths in Grand Rapids rose after fluoridation; however, this was refuted by Swanberg (1953), who found that the opposite was actually true. A report issued by The National Health Federation and published in the *Congressional Record* of July 21, 1975 (as cited in Wolman, 1976), linked cancer mortality patterns in certain U.S. counties to artificial fluoridation of water. In refutation, an exhaustive study of cancer statistics in the United States over a 20-year period (1950 to 1970), made by the National Cancer Institute (as cited in Wolman, 1976), "provides no support for recent claims that fluoridation of water supplies in the U.S. has increased the risk of cancer" and adds that "no significant excess mortality from cancer could be detected up to 15 years after fluoridation in areas where 95% of the population had been abruptly and continuously exposed." In fact, "reduced mortality from cancers of the brain and nervous system in communities with high levels of natural fluoride" was actually found. Doll and Kinlen (1977) reported that a 1976 statement by the Royal College of Physicians that "there is no evidence that fluoride increases the incidence of mortality of cancer in any organ" was challenged on merits of the July 21, 1975. report in the *Congressional Record* (as cited in Wolman, 1976). Doll and Kinlen reexamined the same census data that were evaluated in the report in the *Congressional Record* and found that when age, sex, and ethnic group were taken into account, the ratio between observed cancer mortality and expected cancer mortality actually fell slightly in the cities using fluoridated water supplies. Thus, the National Cancer Institute's refutation of the *Congressional Record* study was reaffirmed. In one other study, Erickson (1978) compared the mortality rates (blacks and whites only) in 24 cities with fluoridated water supplies and 22 cities with nonfluoridated water supplies, and found no evidence of fluoride causing any harmful effects. Other claims of deleterious effects of fluoride from fluoridation of public water supplies were commented on by Rubini (1969) and Bronner (1969) and were shown to be without foundation.

6.4.2.3.2 Vehicles other than water for fluoridation — Vehicles other than water have been suggested for fluoride administration, such as tablets, milk, table salt, or topical application. The question was considered by the Nutrition Committee of the Canadian Pediatric Society (1972), and techniques other than water fluoridation were found to be of interest mainly to special population groups or as a means of supplementation where water of optimum fluoride content was not available. While fluoridation of public water supplies is still the best general way of ensuring adequate intake of fluoride by the general population, changes in food and water intake patterns and increasing use of fluoridated water in food processing make it necessary to monitor the amounts of fluoride people are actually consuming. Prival and Fisher (1974) considered the question and claimed that fluoride intakes are rising. One method of monitoring considered useful is the so-called "market-basket" approach, which is analysis of the fluoride content of a typical two-week diet of a 16- to 19-year-old male. The authors suggested periodic surveys of dental fluorosis in selected artificially fluoridated areas as a basic way of detecting any trend toward increase of fluoride intake above optimum.

6.4.3 Toxicity of Fluorine and Fluorine Compounds

6.4.3.1 Chronic Toxicity, Fluorosis — Chronic toxicity of fluoride is manifested chiefly in the bones where incorporation of fluoride modifies the chemical composition of bone (Weidmann and Weatherell, 1970). Fluoride is a unique ion because it continues to deposit in calcified structures after the other constituents have reached a steady state (Zipkin, 1970). While the calcium-phosphorus ratio remains unchanged, carbonate and citrate are lowered and magnesium increases. A relation between fluoride intake and calcium deposition has been noted by many authors. In spite of the chemical changes, the physiology of the skeleton is not grossly changed by consumption of water containing as much as 8 ppm fluoride (Zipkin, 1970). At about this level on long-term intake, chemical fluorosis may be noted. In its early stages, fluorosis is evidenced by an increased opacity of the bone to X rays.

Roholm (1937*a*) studied workers in the cryolite industry exposed to high levels of fluoride and described the three following stages in the evolution of skeletal fluorosis, as distinguished radiologically: (1) The spinal column and pelvis show roughening and blurring of the trabeculae, or fibrous parts, reflecting both early ectopic calcification and also disorganization in the protein-matrix part of the bone. (2) The trabeculae merge together, and the bone has a diffuse structureless appearance. Contours become uneven, the medullary cavities may be narrowed, and the ligaments show calcification. (3) The bones appear as marble-white shadows with a woolly configuration. The bones of the extremities show irregular periosteal thickening with calcification of ligaments and muscular attachments. The cortex of the long bones is thick and dense, the medullary cavity is diminished, and the interoseous membranes, such as between the tibia and fibula and between the radius and ulna, show calcification.

Singh et al. (1962) studied the skeleton of an individual who had lived in an area of 9.5 ppm water fluoride content. The bones were heavy, irregular, and dull-colored. Multiple exostoses were present, particularly around sites of insertion of muscles and tendons. In the spine, ligaments were calcified and vertebrae were fused at many places and thickened, resulting in pressure on the spinal cord. These changes, and other cases studied showing exostoses around joints, explain the crippling and pain associated with severe fluorosis.

It is paradoxical that the increased calcification is accompanied by bone resorption. Fluoride stimulates both the osteoblasts and the osteoclasts; therefore, bone destruction may proceed side by side with bone formation. Because of this, x-ray pattern may resemble that of Paget's disease and other bone diseases such as osteosclerosis and osteomalacia without elevated fluoride.

It is considered that 4 to 5 mg of fluoride is the daily limit that may be ingested without hazardous body storage (Singh and Jolly, 1970). In areas of endemic fluorosis, levels of ingestion of over 8 mg daily are common, and levels in industrial situations may be 15 to 20 mg daily.

Fluoride levels in bone in fluorotic individuals cover a wide range. It is unlikely that there is a critical threshold of fluorine in the bone for development of fluorosis. (However, a critical threshold would be difficult to determine since the fluoride concentration in bone has to be greater than 5000 ppm before there is an increase in x-ray absorption.) Rather, the level in the bone represents the past history of the individual. If this history involved intake of fluoride such that cellular metabolism was perturbed for a sufficient time, fluorosis resulted. Once in the bones, fluoride causes no problem (see Weatherell, 1966).

Since all the details of bone calcification and metabolism are not known, it is also not known exactly how excess fluoride interferes with normal bone processes. As discussed by Weatherell (1966), fluoride may inhibit enzymes of the bone matrix, may perturb the transport of metabolites across bone cell walls, or may interfere with the synthesis of matrix precursors. Along with this is increased osteogenesis, as evidenced biochemically by increase in serum alkaline phosphatase.

Fand (1973) discussed other aspects of fluorosis in addition to dental and skeletal lesions. On long-standing fluorosis, neurological symptoms may occur that have been designated as a radiculomelopathy by Singh and Jolly (1961). A hypochromic microcytic anemia with defective marrow erythrocyte formation has been noted. There may be increased serum alkaline phosphatase activity, along with increased serum inorganic phosphorus, reduced serum calcium, and increased urinary calcium. Premature aging and signs of hypoadrenocorticalism characterized by decreased urinary 17-ketosteroids may be noted. Digestive disturbances, including a chronic catarrhal gastritis, occur.

Patients with renal insufficiency would not eliminate fluoride as easily as healthy persons and might show signs of fluorosis at lower concentrations than the general population. However, as mentioned in Section 6.3.2.1, fluoride is largely eliminated passively. The consequence of this is that most nephritic conditions do not have much effect on fluoride excretion. Studies of fluoride excretion in normal and nephritic persons and in experimental animals with kidney injury (Hodge, Smith, and Gedalia 1970; Weatherell, 1966) prove this to be true.

The use of fluoridated water for hemodialysis has caused some alarm. Posen et al. (1968, as cited in Aaron 1969) suggested that the fluoridated water (0.9 to 1.0 ppm) of Ottawa, Canada, was a contributing factor to the high incidence of renal azotemic osteodystrophy in 16 patients on long-term hemodialysis. Aaron's discussion (1969), however, discounted this, as osteodystrophy is common in patients with chronic renal failure, including those receiving long-term dialysis with nonfluoridated water. Parsons (1974) considered the question and found no significant differences in fluoride retention in patients dialyzed against fluoridated water in Newcastle and nonfluoridated water in London. There was no convincing correlation between the degree of fluoride retention found in uremic bone and the prevalence of uremic osteodystrophy. Parsons suggested that fluoride in the hemodialysis water may actually be beneficial in opposing the demineralizing process of long-term dialysis. However, Cordy et al. (1974) compared hemodialysis patients using nonfluoridated water with those patients using fluoridated water. In agreement with Posen et al. (1968, as cited in Aaron, 1969) they concluded that patients maintained on long-term hemodialysis (several years) using fluoridated water will show an unacceptable frequency and degree of osteomalacia.

There are several methods of monitoring for fluoride hazards. In most urban areas the fluoride concentration in the drinking water is routinely determined. However, analysis of typical water supplies are required to show that a hazard exists in rural areas. Where persons are exposed to unknown amounts of fluoride, such as dusts and fumes in industrial situations, authorities agree that monitoring of urinary fluoride would provide adequate information concerning fluoride exposure. "Air sampling forms the basis for good industrial hygiene control; urine analysis gives reliable evidence of the effectiveness of the protective program" (Hodge and Smith, 1972). Walbott (1970) criticized this philosophy on the basis of individual variations, but it remains an effective and practical approach when applied systematically.

Threshold limit values for a number of fluoride compounds, including some fluoroorganics, are given in Table 6.13. Most of the compounds for which threshold limit values have been recommended do not present air pollution problems, and many not even workroom problems. In most cases, the industrial exposure concentrations are significantly below these values.

TABLE 6.13. THRESHOLD LIMIT VALUES OF VARIOUS FLUORINE COMPOUNDS

Fluoride-containing substance	ACGIH[a] threshold limit value		Pennsylvania short-term limit
	(ppm)	(mg/m^3)	
Boron trifluoride[b]	1	3	1 ppm for 5 min
Bromine pentafluoride	0.1	0.7	
Chlorine trifluoride[b]	0.1	0.4	0.1 ppm for 5 min
Dichlorodifluoromethane	1000	4950	
Dichloromonofluoromethane	1000	4200	
Dichlorotetrafluoroethane	1000	7000	
Difluorodibromomethane	100	860	
Fluoride (F^-)		2.5	10 mg/m^3 for 30 min
Fluorine	0.1	0.2	0.5 ppm for 5 min
Fluorotrichloromethane	1000	5600	
Hydrogen fluoride[b]	3	2	3 ppm for 15 min
Nitrogen trifluoride	10	29	
Oxygen difluoride	0.05	0.1	
Perchloryl fluoride	3	13.5	
Selenium hexafluoride	0.05	0.4	
Sodium fluoroacetate (1080)		0.05	
Sulfur hexafluoride	1000	6000	
Sulfur pentafluoride	0.025	0.25	
Sulfuryl fluoride	5	20	
Tellurium hexafluoride	0.02	0.2	
1,1,1,2-Tetrachloro-2,2-difluoroethane	500	4170	
1,1,2,2-Tetrachloro-1,2-difluoroethane	500	4170	
1,1,2-Trichloro-1,2,2-trifluoroethane	1000	7600	
Trifluoromonobromomethane	1000	6100	

[a] American Conference of Governmental Industrial Hygienists.
[b] Ceiling value — a value not to be exceeded even briefly.

Source: Reprinted from *Fluorides*, Publ. 1922, p. 231, with the permission of the National Academy of Sciences, Washington, D.C.

As noted, the threshold limit value for inorganic fluoride is 2.5 mg/m^3. A few countries consider this too high and recommend 1.0 mg/m^3 instead. The countries included are the Soviet Union, Poland, Hungary, Czechoslovakia, and East Germany. It should be noted, however, that the philosophy of the Soviet Union hinges on finding the smallest dose or the lowest concentration that produces a reproducible effect of any sort; in the case of hydrogen fluoride, for example, the results of a test using a transmitted impulse from the olfactory nerve of a dog inhaling hydrogen fluoride in air were used. An air-quality standard for fluoride of 0.01 mg of fluoride per cubic meter for a 24-hr average concentration with a maximum allowable single peak concentration of 0.03 mg/m^3 in the ambient air of inhabited areas, has been established in the Soviet Union and several other eastern countries (National Academy of Sciences, 1971, pp. 230-231). The National Academy of Sciences document questions the practicality of such low limits and considers them more representative of a final goal to be achieved than a true reflection of an existing situation.

Zielhuis (1974) discussed differences between the United States and the Soviet Union in their approach to setting permissible limits for respiratory exposure to chemical agents in industry. In the United States, research is often focused on cells or organs; in the U.S.S.R. the emphasis is on central nervous system effects (Ryazanov, 1962). Thus, differences arise in interpretation of biological changes regarding health. Zielhuis (1974) made a plea for exchange of basic data because the two approaches clearly complement each other.

For the general population, water is the chief source of fluoride. The close relationship between fluoride concentrations in water and in urine is shown in Figure 6.24. Largent (1961) proposed that an average urinary excretion of at least 8 mg of fluoride per liter over a period of more than five years would be required to corroborate a diagnosis of skeletal fluorosis. This prediction was supported by the findings of Sankaran and Gadukar (1964). They found levels of urinary fluoride excretion of 8 to 22 ppm in 6 cases of early to advanced human skeletal fluorosis.

The mobilization of fluoride as evidenced by continued urinary excretion on withdrawal of fluoride was mentioned in Section 6.3.2.2. Such excretions chiefly result from the osteoclastic activity of normal bone cycline (Hodge and Smith, 1965). Fluorotic patients brought to a normal regime showed that the release of fluorine from the bone into the blood and thence into the urine slowly declined over a period of several years — half-life of eight to ten years (Forbes et al., 1978).

A simple diagram of bone dynamics is given in Figure 6.25. At doses below poisoning levels and below levels where crippling fluorosis may ensue, fluoride stimulates bone formation, induces partial resorption, and causes changes in the degree of mineralization. Since bone formation prevails over enhancement of resorption, total bone mass increases. New bone, rich in fluoride, resists osteoclastic action because of the decreased solubility of fluorapatite as compared with hydroxyapatite. Feedback from the process results in a secondary hyperparathyroidism. This leads to osteoblastic as well as osteoclastic differentiation, the former causing increased chondrogenesis and matrix formation. The high organic content of bone thus formed accords with the observation, made histologically, that the exostoses of fluorosis resemble fetal bone, where high osteoblastic activity is of course normal. The alterations of normal growth and development produced by excess fluoride were summarized by Hodge and Smith (1965) as: (1) acceleration of cortical remodeling, (2) dissociation of the normal sequences in osteogenesis, and (3) the production of abnormal bone.

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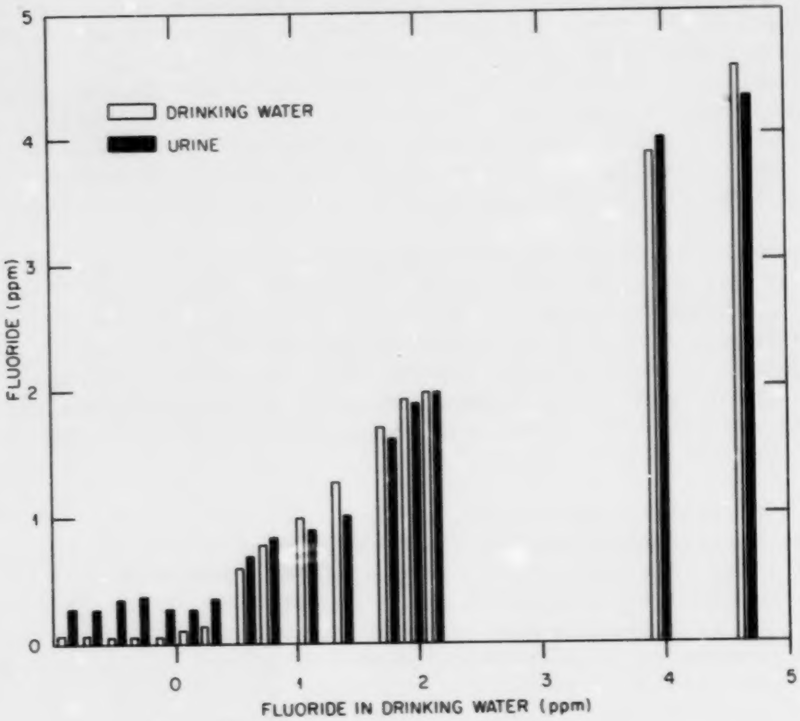

Figure 6.24. Relationship between fluoride concentration of drinking water and fluoride concentration of the urine. Source: Adapted from McClure and Kinser, 1944, Figure 1, p. 1582. Reprinted by permission of the publisher.

ORNL-DWG 79-20910

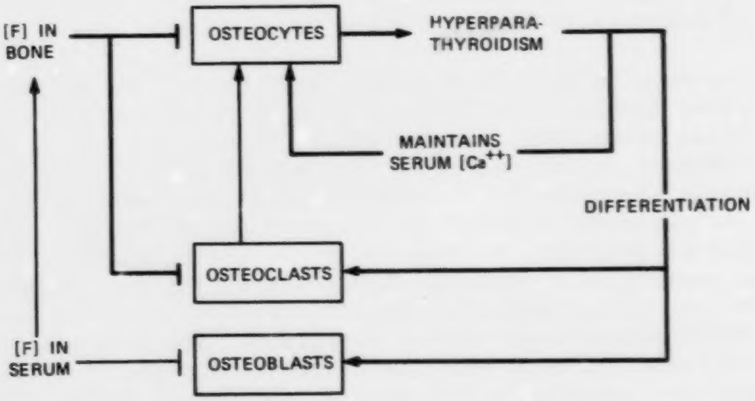
[F] IN BONE
OSTEOCYTES
HYPERPARA-THYROIDISM
MAINTAINS SERUM [Ca^{++}]
DIFFERENTIATION
OSTEOCLASTS
[F] IN SERUM
OSTEOBLASTS

Figure 6.25. Scheme of the effects of fluoride on calcium homeostasis (osteocytic resorption) and bone metabolism (osteoclastic resorption). The lines ending in a perpendicular bar indicate inhibition; those ending in an arrow indicate stimulation. Source: Rich and Feist, 1970, Figure 4, p. 82.

Fluoride effects on enzymes were discussed in Section 6.4.1. Roholm (1937*a*) in his treatise on fluoride intoxication, pointed out that the enzyme system most susceptible to fluoride is a lipase. Hodge and Smith (1965) mentioned the findings of several authors: Leake et al. (1929) noted that hepatic lipase activity was suppressed by in vivo intravenous fluoride injections of 75 to 150 mg/kg fluoride, and Sievert and Phillips (1959) reported curtailment of renal fatty acid oxidase activity by in vivo fluoride concentrations of 0.1% and 0.15% of the diet feed. Burstone and Keyes (1957) demonstrated that a fatty acid, esterase, was the chief enzyme associated with bone formation, affected by in vivo sodium fluoride injections of 0.1 ml of 0.05 *M* solution twice a day for three days. The studies of Johnson (1960) on mineralization of collagen showed involvement of phospholipids in the chemistry of the osteoblasts and in the transformation of osteoid material to bone. A constant finding of extensive serous atrophy of the fatty marrow in the bones of animals maintained on very high fluoride diets (900 ppm for 71 days) (Hodge and Smith, 1965) further showed the importance of lipids in bone metabolism.

The osteosclerotic effect of fluoride and research done on wound and fracture healing in the presence of fluoride have stimulated studies on fluoride use in therapy of bone disorders. Gedalia and Zipkin (1973) reviewed the question and concluded that fluoride therapy must still be considered experimental because much work remains to be done on establishing optimal dosages, on coupling fluoride therapy with other medications (e.g., vitamins and hormones), and in the elaboration of fluoride compounds more specific and less toxic than simple fluorides. On balance, however, it appears that fluoride-induced osteosclerotic bone, although not as satisfactory as original bone, is preferable to osteoporotic bone. According to Hodge and Smith (1968), the benign nature of early skeletal fluorosis and the mildness of the adverse reactions from fluoride doses as high as 60 mg daily make the risks of fluoride treatment acceptable.

An optic neuritis is, however, a possible contraindication. Neer et al. (1966) studied a patient with relatively stabilized multiple myeloma with generalized osteoporosis and found a beneficial effect (progressively positive calcium, presumptive stabilization of bone crystal) over three years at a fluoride dose of 27 mg daily. Carbone et al. (1968) found that administration of fluoride following, or concurrent with, antineoplastic treatment produced some benefit in myeloma patients with bone lesions. Similarly, Gompels, Votaw, and Martel (1972) found some beneficial increase in bone density in some patients with multiple myeloma who were receiving fluoride therapy. In contrast, Harley, Schilling, and Glidewell (1972), in a double-blind study of 150 patients with multiple myeloma, found no benefit from fluoride treatment and even a suggestion that production of fluorosis might be detrimental to myeloma patients because the new, low-quality, somewhat disorganized bone being laid down would be an inroad for the myelomatous process.

The use of fluoride is probably of more benefit in developmental and degenerative or metabolic bone disorders than in neoplastic-associated ones. Whereas Albright and Grunt (1971) found only marginal benefit of fluoride in 20 children and 5 adults with osteogenesis imperfecta, Bernstein et al.

(1963) found increased calcium retention, increased mineralization, and some relief of bone pain in patients with postmenopausal osteoporosis, urticaria pigmentosa with osteoporosis, and idiopathic osteoporosis. These patients received doses of sodium fluoride of 50 to 200 mg/day (22 to 90 mg of fluoride). Bernstein et al. (1966) also found a lower prevalence of osteoporosis, collapsed vertebrae, and back pain in residents over age 45 (particularly women) in a community of high fluoride water concentration (4.0 to 5.8 ppm) as compared with residents of a community having a low concentration of fluoride in the water (0.15 to 0.3 ppm). The male subjects, however, showed little difference in the incidence of spontaneous collapsed vertebrae as a function of fluoride water concentration. The 4.0 to 5.8 ppm level is, however, high for the general populace because of the potential enamel defects (mottling) on developing teeth.

As seen in Figure 6.25, imbalance in bone formation on stimulation with fluoride may arise from lack of calcium. Jowsey et al. (1972) found that twice weekly administration of a combination of 50 mg of sodium fluoride, 50,000 units of vitamin D, and 900 mg of dissolved calcium was beneficial in the treatment of osteoporosis. Zanzi et al. (1975) found increases in skeletal mass in some, but not all, osteoporotic patients on treatment with salmon calcitonin, sodium fluoride, and dissolved calcium.

Shambaugh and Scott (1964) used sodium fluoride to induce or hasten maturation and inactivation of otosclerotic foci in ear bones in order to arrest progression in conductive and sensorineural hearing loss.

6.4.3.2 Acute Toxicity — A reasonable estimate of a "certainly lethal dose" of sodium fluoride for a 70-kg man is 5 to 10 g (Hodge and Smith, 1965). This corresponds to 70 to 140 mg/kg. Since sodium fluoride is 19/42 fluoride by weight, this corresponds to 2.2 to 4.5 g of fluoride, or 32 to 64 mg per kilogram of body weight. Roholm (1937*a*) reported 4 g of sodium fluoride as the smallest dose to cause death in an adult man.

Sodium fluoride used as a roach and ant powder may be carelessly kept near food and mistaken for sugar or flour. Incidents of poisoning have occurred this way. Pure sodium fluoride is white, but federal law now requires that it be colored blue or green (National Academy of Sciences, 1971); nonetheless, accidents still occur. Other fluoride compounds are also used as insecticides and rodenticides; they are as poisonous as sodium fluoride on the basis of the amount of fluoride absorbed. Metcalf (1966) listed the toxicities to insects of a number of inorganic fluoride compounds. The toxicities generally are a function of the fluoride content. To humans, the toxicity depends on the ease of absorption of the compound. Thus, cryolite (Na_3AlF_6), which has a relatively low solubility and is a natural product, has been used as a spray or as a dust for the control of chewing insects. It is relatively innocuous to foliage and has a low toxicity to mammals; the oral LD_{50} in the rat is approximately 13,500 mg/kg.

Other fluorine-containing compounds in the home taken as recommended (e.g., anticaries tablets, some pediatric formulations, and toothpaste) are not hazardous. Hodge (1969), however, cautioned that some commercially available rust removers contain dangerous quantities of hydrofluoric acid.

Because of the resulting massive availability, fluoride compounds are lethal at considerably lower doses when injected rather than ingested. Thus, the 24-hr intraperitoneal LD_{50} of sodium fluoride for rats was 23 mg/kg, about one-fifth of the oral LD_{50}. Showing the importance of solubility, the 24-hr LD_{50} for cryolite given intraperitoneally in rats was 25,000 mg/kg, while for calcium fluoride it was 1500 mg/kg (Hodge and Smith, 1965).

The amount of fluoride absorbed into soft tissues seems to be the most critical point in fluoride poisoning. Gettler and Ellerbrook (1939) analyzed remaining fluoride in the internal organs of five persons fatally poisoned. They estimated from these analyses that 105 mg is the smallest absorbed lethal dose of fluoride in a 140-lb individual. On the basis of known patterns of fluoride distribution, Hodge and Smith (1965) estimated that the 105 mg of fluoride would represent 5% of the ingested dose, which would have been 4 to 5 g of sodium fluoride. The 105 mg is the fraction of the total absorbed fluoride remaining in the soft tissues at death. They suggested that this be called the "smallest soft tissue burden."

There is no single cause of death in fluoride poisoning. Respiratory blockage is certainly important but is not the only major effect. Hodge (1969) grouped most of the acute fluoride effects into four categories of major functional derangements: (1) enzyme inhibition, (2) calcium complex formation, (3) shock, and (4) specific organ injury. Effects of fluoride on various enzymes and enzyme systems are discussed in Section 6.4.1. At acute levels these inhibitions may be contributory to death. Hodge and Smith (1965) summarized the effects of the four categories. In acute poisoning, fluoride kills by blocking normal cellular metabolism. Fluoride inhibits enzymes involved in essential processes, causing vital functions, such as the origin and transmission of nerve impulses, to cease. Interference with necessary body functions controlled by calcium (e.g., blood clotting and membrane permeability) may be equally important. Massive impairment in the function of vital organs results from cell damage and necrosis. Terminally there is a characteristic shock-like syndrome.

Fatal poisoning is rapid, death frequently occurring in 2 to 4 hr (Hodge and Smith, 1965). When soluble fluoride is ingested, a catastrophic series of events is set in motion. There is salivation, a burning sensation, nasal discharge, nausea, mucosal irritation, urination, vomiting with blood, and diarrhea with blood. The pH of the stomach causes some hydrogen fluoride to be formed. When this is regurgitated, extreme irritation of the esophagus, throat, and upper respiratory tract ensues. There is urticaria, muscular weakness, excitement and tremor, convulsion, and fall of blood pressure (Greenwood, 1940). Initially there is an acceleration and deepening of respiration that is followed by a general paralysis of the vital nervous centers. Death occurs by general collapse; shock, involving tetany, is a prominent characteristic. On autopsy, general congestion of the organs is noted, with renal tubular degeneration. The blood is slow to clot because of the calciprivic action of fluoride.

Polson and Tattersall (1969) described a number of cases of fluoride poisoning — suicidal, homicidal, and accidental. One woman died in 15 min after taking 17.2 g of sodium fluoride. Numerous cases of fluoride poisoning occurred in a mental hospital in Oregon (Lidbeck, Hill, and Beeman, 1943). Roach powder was mistaken for powdered milk, added to scrambled eggs, and served to patients in five wards. Some rejected the dish because of a salty or soapy taste. Of 263 patients who ingested the fluoride, 47 died.

Fluoride poisoning has no particular or special signs but resembles poisoning from other violent gastrointestinal irritants, notably arsenic, mercury, barium, and oxalic acid (Polson and Tattersall, 1969). It has been mistaken for botulism. There is no specific treatment, except for administration of calcium salts. Vomiting is usually spontaneous; if not, an emetic should be given. Gastric lavage with limewater is effective. Calcium chloride or milk by mouth may be given. A soluble calcium salt may be given intravenously; calcium gluconate is often used. Because the kidneys can excrete a great deal of fluoride and some fluoride is taken up by the bones, if a patient survives the first few hours of poisoning, chances of survival are good with supportive measures. Abukurah et al. (1972) reported the case of a man who survived after ingesting 120 g of sodium fluoride in a suicide attempt. Nausea and vomiting occurred immediately and persisted for 1 hr prior to hospitalization. Tetanic contractions and ventricular fibrillation, requiring countershock and cardiac massage, were features of the case. Fluid therapy and administration of diuretics to protect against tubular necrosis and to increase excretion of fluoride were effectively used to remove the patient from the acute phase of poisoning. The patient recovered without sequelae. This is generally the case in recovery from fluoride poisoning. Thus, except for necrosis and burns, as in the case of exposure to hydrogen fluoride, the effects of fluoride appear to be mainly reversible.

6.4.3.3 Toxicity and Hazards of Inorganic Fluoride Compounds

6.4.3.3.1 Hydrogen fluoride and hydrofluoric acid — Exposures to hydrogen fluoride, F_2, and hydrofluoric acid (SiF_4) usually occur in industry. Poisoning by hydrogen fluoride is rare. Although it is highly dangerous because of its fluorine content and corrosive action, hydrogen fluoride's extremely irritating nature serves as a warning. Used in water solution, it can be extremely dangerous. It penetrates the skin easily, resulting in deep burns that develop into abcesses. Hydrogen fluoride is as toxic as other fluorides on the basis of fluorine content, but in cases of inhalation (as a gas or as vapors of an aqueous solution) the respiratory inflammation and pulmonary edema due to its irritant nature tend to overshadow the general aspects of fluoride poisoning mentioned in the preceding section. This is shown by the outcome of two cases described by Greendyke and Hodge (1964). Two men at work noticed a bottle of hydrofluoric acid emitting fumes. The men attempted to remove the bottle, but it exploded, showering them with acid and enveloping them in dense fumes. Fellow workmen sprayed them with a fire hose, and a physician washed the burns with bicarbonate and administered an opiate. The men were then taken to a hospital. After 4 hr, one man died from intense inflammation of his respiratory tract and gross hemorrhagic pulmonary edema, in spite of a tracheotomy. An autopsy showed bloody mucus in the stomach as well as the lungs; the fluoride blood level was 4 ppm. The other man died after 10 hr from cardiac arrest and apnea. Autopsy findings were similar to those of the other victim.

Because these men were 85 miles from a hospital and several hours elapsed before they were treated, it is possible they might have survived with earlier treatment; however, it is difficult to know what to do to counteract lung damage. Mayer and Guelich (1963) estimated that hydrogen fluoride contamination from splashes of concentrated hydrofluoric acid, particularly in the chest area and breathing zone, could produce concentrations of 10^4 to 10^5 ppm that might do irreversible, fatal damage to lung tissue in less than a minute. It may be noted that hydrofluoric acid is used in industry as an aqueous solution in concentrations up to 70% and as a pure liquid under pressure. The vapor pressures of aqueous solutions are such that an inhalation hazard probably exists only with solutions containing more than 60% hydrogen fluoride (Mayer and Guelich, 1963). Enough hydrogen fluoride can be absorbed through the skin that systemic fluoride poisoning results. This is shown in a case studied by Burke, Hoegg, and Phillips (1973). In a laboratory accident, a technician's face, neck, and arm were sprayed with liquid hydrogen fluoride. The man was placed in a shower and washed for 15 min. In the hospital, under general anesthesia, the skin burns were infiltrated with calcium gluconate. The patient showed signs of general fluoride poisoning, with stupor and polyuria. Urine fluoride analyses showed he had absorbed a total systemic dose of at least 404 mg of fluoride from an estimated 5 g of hydrogen fluoride spilled on about 2.5% of his body surface. The patient recovered without sequelae, except for skin burns which were repaired by plastic surgery.

6.4.3.3.2 Elemental fluorine — Fluorine is more toxic than hydrogen fluoride. The U.S. safe standard (Hamilton and Hardy, 1974) for exposure to hydrogen fluoride is 3 ppm compared to 0.1 ppm for fluorine. For fluoride salts in air (dusts and fumes) it is 2.5 mg/m^3. Fluorine causes burns, as does hydrogen fluoride. As described by Dreisbach (1971), inhalation of hydrogen fluoride or fluoride causes coughing, choking, and chills lasting 1 to 2 hr after exposure. Over one or two days, fever, coughing, chest tightness, rales, and cyanosis develop, indicating pulmonary edema. The symptoms progress for a day or two and then regress slowly over a period of a few weeks.

Some exposure limits have been given in the Hygienic Guide series of the American Industrial Hygiene Association (1965). The maximum atmospheric concentration for an 8-hr exposure, as determined by experiments with animals, was 0.1 ppm. Short exposure tolerance, as observed in two men exposed very briefly, was 25 ppm. This caused sore throat and chest pain for 6 hr. The volunteers felt they could not tolerate 50 ppm. One ppm for 1 hr was not expected to cause injury. Atmospheric concentrations immediately hazardous to life are not known for humans, but test animals have died at concentrations ranging from 10,000 ppm for 5 min to 200 ppm for 3 hr. Keplinger and Suissa (1968) found only slight irritation upon exposure of volunteer human subjects to 25 ppm but marked irritation of eyes and nose at 100 ppm. Largent (1949) gave the progression of effects with concentrations of gaseous fluorides (e.g., hydrogen fluoride and F_2 giving rise to hydrogen fluoride). No local immediate systemic effects were noted at 3 ppm, but at 10 ppm many persons experienced discomfort. At 20 ppm, all persons complained and objected seriously to staying in the environment. Brief exposures to 60 ppm caused definite irritation of the conjunctivae and nasal passages and tickling and discomfort of the pharynx and trachea. The highest concentration tolerated was 120 ppm for less than 1 min by two men.

Some effects of fluorine are qualitatively different from those of neutral fluorides or of hydrogen fluoride. Hodge and Smith (1965) suggested that perhaps it is not F_2 but some highly reactive product (e.g., OF_2) that is responsible for the respiratory irritation when F_2 is released in air.

Ricca (1970) reviewed the pathotoxicology of elemental fluorine, with particular attention to the physiochemistry of fluorine reactions with animal proteins and lipids. Fluorine can react directly with organic compounds to yield free radicals without the requirement of an initiator. Disruption of the fluorine molecule gives hydrogen fluoride, a free-radical fluorine atom, and a free-radical organic moiety. Addition of fluorine across double bonds is also an important feature of fluorine reactivity. Some C-F and N-F fluorosubstituted compounds are potent competitive inhibitors of natural substrates or may take the place of natural organic molecular building blocks. Chemical bonds may be broken by fluorine attack, causing aberrations in metabolic chains. Finally, fluorine elimination shows differences from that of fluorides. Ricca attributed the effects of fluorine to formation of hydrogen fluoride and fluoride, plus the special effects of fluorine itself. On the basis of his own work and a survey of the literature, Ricca suggested the following limits: an emergency exposure limit of 25 ppm for 5 min, an emergency tolerance limit of 15 ppm for 10 min, and a threshold limit value of 1.0 ppm for 8 hr.

6.4.3.3.3 Silicon tetrafluoride — Silicon tetrafluoride, used to make fluorinated compounds, is gaseous and has effects and toxicity similar to those of hydrogen fluoride (Greenwood, 1940). Hydrofluorosilicic acid and soluble salts have actions similar to those of hydrofluoric acid and fluorides. Roholm (1937*b*) analyzed a disastrous fog that occurred in the Meuse Valley near Liege, Belgium, in 1930. He concluded that the disaster may have been due to acute fluoride poisoning that resulted from gaseous fluorine compounds (HF, SiF_4) liberated by certain factories in the area in conjunction with an unusually dense fog. Heavy fumes of fluorosilicic acid are generated from SiF_4 in moist air. Several thousand persons suffered acute pulmonary attacks, and 60 deaths were reported.

6.4.3.4 Fluorine in Pesticides — Fluoride sprays were introduced in the early decades of this century partly to replace arsenate sprays with their residue problems, particularly lead arsenate. Use was considerable at one time, and sodium fluoride, sodium and barium fluorosilicates, and cryolite were used most extensively (Hodge and Smith, 1965). However, by the late 1960s, more effective insecticides became available, and the use of all types of fluoride insecticides virtually ceased (National Academy of Sciences, 1971). In view of the relatively small amounts now used, it does not appear that there is widespread hazard from either inorganic or organic fluorine-containing insecticides.

6.4.3.5 Toxic Plant Fluorocarbon, Lethal Synthesis — Some plants synthesize fluoro compounds that are toxic or that become toxic by metabolic conversion (lethal synthesis). The first discovered was fluoroacetate (Marais, 1944), which is found in leaves of the plant *Dichapetalum cymosum* in South Africa. Peters (1972) cited the number of plants known to contain fluoroacetate at about 36. Fluorooleic acid, with smaller amounts of shorter chain fluoro acids (Peters, 1972), are found in West Africa in the seeds of *Dichapetalum toxicarium*. Microorganisms can process these fluoro compounds to make, for instance, fluoromalonate from fluoroacetate (Pattison and Peters, 1966), or to degrade the compound completely. The toxicity of the compounds in animals is due to conversion to fluorocitrate, which blocks the tricarboxylic metabolic cycle. A "delayed convulsive action" results. The compounds are very toxic: Pattison and Peters (1966) estimated the lethal dose of fluoroacetate in man to be between 5 and 20 mg/kg. Pattison (1959) discussed other cases of human poisoning. Peters (1972) stressed the need for finding an antidote to poisoning by fluoroacetate and similar compounds. Fluoroacetate is a commerical rodenticide and insecticide and could present a danger to humans if used indiscriminately. Yu and Miller (1970) reported the presence of fluoroacetate in a few samples of crested wheat grass collected near a phosphate factory in Montana. Cheng et al. (1968) and Lovelace et al. (1968) reported that cultivated plants such as crested wheat grass and soybeans may have a certain amount of fluoroacetate and fluorocitrate, the amounts found, however, being too small to present a hazard.

6.4.3.6 Fluorine Compounds, Including Fluoroorganics — Rudge (1962) mentioned that the physiological properties of fluorine and its compounds cover the complete gamut, from the innocuous through the beneficial to the extremely toxic. The saturated fluorocarbons can be classified in the innocuous range. Some of these, such as Teflon (polytetrafluoroethylene), are among the most inert chemical substances known. There is a great deal yet to be learned about the relationship between chemical constitution and physiological behavior of fluorine-containing compounds. Fluothane ($CF_3CClBrH$) is a potent anesthetic with relatively few side effects, whereas 2,2'-hexafluorodiethyl ether ($CF_3CH_2OCH_2CF_3$) causes convulsions when inhaled. While tetrafluoroethylene ($CF_2{=}CF_2$) appears to be nontoxic, most of the higher unsaturated fluorocarbons and chlorofluorocarbons are very toxic. Sulfur pentafluoride (S_2F_{10}) is extremely toxic, causing pulmonary edema. However, sulfur hexafluoride is so inert it may be breathed in 80% concentration by volume when mixed with oxygen with no adverse physiological effects except for a slight narcosis (Rudge, 1962).

Clayton (1966) reviewed the mammalian toxicology of organic compounds containing fluorine. Cook and Pierce (1973) noted the tendency of unsaturated fluoro compounds to be toxic and considered the mechanism of this effect. Table 6.14 shows data collected on the toxicities of some chloroolefins and fluoroolefins. While admitting some toxic effect due to hydrolysis, Cook and Pierce (1973) showed that the toxicity of halogenated olefin directly reflects its reactivity to nucleophiles. Thus, substitution of fluorine for hydrogen makes the double bond in the compound $(CF_3)_2C=CF_2$ more susceptible to nucleophilic attack than the double bond in the compound $CF_2=CF_2$, and the toxicity follows the same pattern. The chief toxicity of the fluoroolefins is apparently due to their activity as biological alkylating agents. The authors warn that whenever one works with a polyfluorinated olefin of high nucleophilic susceptibility and there is an absence of specific toxicological information, extraordinary safety precautions should be taken.

6.4.3.6.1 Toxicities of propellants and refrigerants — Table 6.15 shows the results of tests done mainly in the 1930s on the acute inhalation toxicity of various fluoromethanes. The compounds were developed as fire-extinguishing agents or refrigerants, and therefore the test design featured exposure of up to 2 hr only. As seen, the fluorinated compounds are generally in the high Underwriters' Laboratories (UL) class (i.e., show low toxicity).

TABLE 6.14. OLEFIN TOXICITIES

Olefin	LC_{50}[a] (ppm)	ALC[b] (ppm)
$CH_2=CHF$		800,000
$CH_2=CF_2$		128,000
$CF_2=CF_2$	40,000	
$CH_2=CCl_2$	32,000	
$CHCl=CCl_2$		8,000
$CCl_2=CCl_2$		4,000
$CF_3CF=CF_2$	3,000 3,240-13,365 (2 hr)	
$CF_2=CFCl$	1,000 7,560 (2 hr)	
$CF_2=CCl_2$		1,000
$CF_3CF=CHCF_3$		200
$CF_3CCl=CClCF_3$		100
trans	61 (1 hr	
cis	179 (1 hr)	
$(CF_3)_2C=CF_2$	0.76	

[a] Lethal concentration for 50% of animals, 4-hr exposure.

[b] Approximate lethal concentration, 4-hr exposure.

Source: Adapted from Cook and Pierce, 1973, Table 1, p. 337. Reprinted by permission of the publisher.

TABLE 6.15. INHALATION TOXICITY OF FLUOROMETHANES

Group	Structure	Exposure: Concentration (%)	Exposure: Time (hr)	Fatal	UL[a] class	TLV[b]
A	$CHCl_3$	2.0	2	+	3	50
	$CHCl_2F$	10.0	1	+	4-5	1000
	$CHClF_2$	20.0	2	-	5a	(1000)
	CHF_3	20.0	2	-	6[c]	(1000)
B	CCl_4	2.0	1	+	3	10
	CCl_3F	10.0	2	-	5a	1000
	CCl_2F_2	20.0	2	-	6	1000
	$CClF_3$	20.0	2	-	6[c]	(1000)
	CF_4	20.0	2	-	6[c]	(1000)
C	CH_3Cl	2.0	2	+	4	100
	CH_2Cl_2	5.0	2	+	4-5	500
	$CHCl_2F$	10	1	+	4-5	1000
	CCl_2F_2	20	2	-	6	1000

[a] Underwriters' Laboratories classification; the higher the value, the lower the toxicity.

[b] Threshold limit value assigned by the American Conference of Governmental Industrial Hygienists, 1964 values; figures in parentheses indicate provisional values.

[c] Based on data from Haskell Laboratory.

Source: Adapted from Clayton, 1967, Table I, p. 338. Reprinted by permission of the publisher.

Clayton remarked that the "1000 ppm" level is somewhat arbitrary. Values given by Cook (1945) ranged from 5,000 ppm for dichlorofluoromethane to 100,000 ppm for dichlorodifluoromethane. The values were subsequently lowered to 1000 ppm, not because of toxicity but on the premise that good engineering practice demands that no environmental contaminant should be allowed to exceed 1000 ppm (8-hr average). Only carbon dioxide has a threshold limit value greater than 1000 ppm, namely, 5000 ppm.

Since the early studies of fluorocarbons, considerable work has been done on the solubilities in blood, pharmacokinetics, and long-term toxicities of fluorocarbons. Thus Dolley et al. (1970) and Paterson, Sudlow, and Walker (1971) studied the blood levels of fluorinated hydrocarbons in normal and asthmatic individuals after inhalation of pressurized aerosols. The aerosols are used to deliver adrenergic bronchodilator drugs, and the medication is often overused. Another abuse is inhalation of aerosols to become mildly stimulated and intoxicated. Deaths have resulted from this practice, apparently due to cardiac arrhythmia caused by the sensitizing effect of the fluorocarbons to circulating catecholamines (Bass, 1970). The propellants were found in the blood after inhalation, but in considerably lower concentration than when fluorocarbons were used as anesthetics. It was felt that even with overuse there was no great hazard. The fluorocarbons are fat-soluble, accounting for the degree of absorption observed.

Chiou and colleagues studied the absorption, distribution, and elimination of fluorocarbon aerosol propellants in dogs (Chang and Chiou, 1976;

Niazi and Chiou, 1976), humans, monkeys, rats, and mice (Niazi and Chiou, 1975). The propellants were found to have a longer biological half-life than previously thought and to undergo extensive distribution in the body. Results with dogs on intravenous injection of a single dose of fluorocarbon, in this instance dichlorotetrafluoroethane, showed a three-compartment distribution, with half-lives of 1.3, 9.6, and 50.8 min, respectively, for the initial, intermediate, and final disposition phases. Two hours were required to achieve pseudo distribution equilibrium. There was a triexponential decay curve of fluorocarbon in the blood, and 84% of the propellant was cleared from the blood passing through the lungs in each cycle. Compartments were considered to be based on whether the tissues were vessel-rich or vessel-poor, on fat solubility of the fluorocarbon, and on interactions such as protein binding and other complexations. The retentions found have implications for the toxicity profiles of fluorocarbons.

Aviado (1974) reviewed the toxicity of 15 propellants used in aerosols. Chronic and acute inhalation toxicity, acute cardiovascular toxicity (e.g., cardiac arrhythmias and myocardial depression), and acute bronchopulmonary toxicity were the chief reported effects. Propellants were divided into four categories (Table 6.16). The classifications are, in some instances, more severe than the previous UL ratings (Table 6.17).

TABLE 6.16. CLASSIFICATION OF PROPELLANTS BASED ON TOXICITY TO RESPIRATORY AND CIRCULATORY SYSTEMS

Name of propellant	Fluorocarbon number	Underwriters' Laboratories classification	Frequency of use in aerosols
Low-pressure propellants of high toxicity			
Trichlorofluoromethane	FC 11	5a	93
Dichloromonofluoromethane	FC 21	4-5	
Trichlorotrifluoroethane	FC 113	4-5	
Trichloroethane		4-5	1
Methylene chloride		4-5	8
Low-pressure propellants of intermediate toxicity			
Dichlorotetrafluoroethane	FC 114	6	36
Monochlorodifluoroethane	FC 142b	5a	2
Isobutane		5b	37
Octafluorocyclobutane	FC C-318	6	
High-pressure propellants of intermediate toxicity			
Dichlorodifluoromethane	FC 12	6	153
Monochlorodifluoromethane	FC 22	5a	
Propane		5b	6
Vinyl chloride		4-5	1
High-pressure propellants of low toxicity			
Chloropentafluoroethane	FC 115	6	
Difluoroethane	FC 152a	6	5

Source: Adapted from Aviado, 1974, Table 2, p. 371. Reprinted by permission of the publisher.

TABLE 6.17. COMPARATIVE LIFE HAZARD OF GASES AND VAPORS

Group number and definition	Not used as propellants	Used as propellants	
		Low pressure	High pressure
Group 1: Gases or vapors which in concentrations of 0.5% to 1.0% for durations of exposure of 5 min are lethal or produce serious injury	Sulfur dioxide		
Group 2: Gases or vapors which in concentrations of 0.5% to 1.0% for duration of exposure of 30 min are lethal or produce serious injury	Ammonia Methyl bromide		
Group 3: Gases or vapors which in concentrations of 2.0% to 2.5% for duration of exposure of 1 hr are lethal or produce serious injury	Carbon tetrachloride Chloroform Methyl formate		
Group 4: Gases or vapors which in concentrations of 2.0% to 2.5% for duration of exposure of 2 hr are lethal or produce serious injury	Dichloroethylene Methyl chloride Ethyl bromide		
Between groups 4 and 5: Gases or vapors less toxic than group 4	Ethyl chloride	Trichloroethane[a] Methylene chloride Trichlorotrifluoroethane (FC 113) Dichlorofluoromethane (FC 21)	Vinyl chloride[a]
Group 5a: Gases or vapors much less toxic than group 4 but more toxic than group 6	Carbon dioxide	Trichlorofluoromethane (FC 11) Monochlorodifluoroethane (FC 142b)	Monochlorodifluoromethane (FC 22)
Group 5b: Gases or vapors which available data indicate would be classified as either group 5 or group 6	Ethane	Isobutane	Propane
Group 6: Gases or vapors which in concentrations up to at least 20% for duration of exposure of 2 hr do not appear to produce injury	Chlorotrifluoromethane (FC 13)	Dichlorotetrafluoroethane (FC 114) Octafluorocyclobutane (FC C-318)	Dichlorodifluoromethane (FC 12) Difluoroethane (FC 152a) Chloropentafluoroethane (FC 115)

[a] Not tested by Underwriters' Laboratories but estimated to belong in the group.

Source: Adapted from Aviado, 1974, Table 1, p. 368. Reprinted by permission of the publisher.

For instance, octafluorocyclobutane, given a UL rating of 6, appears in the "low-pressure propellants of intermediate toxicity" category in the proposed classification. The hazards of fluoro compounds and of other gases and vapors are compared in Table 6.17.

Alarie, Choby, and Poel (1972) observed alveolar instability and death in rats following intravenous administration of 0.5 to 0.8 ml of tetrachlorodifluoroethane (TCDF). Because of its immiscibility with water, low surface tension, and ability to solubilize lipids, TCDF is apparently able to remove the surfactant normally present on the alveolar surface, with consequent exudation and alveolar collapse. Although the experimental conditions were admittedly severe, they nevertheless indicated the danger due to the fat-solubilizing nature of the fluorocarbons.

In contrast with the study of Alarie, Choby, and Poel (1972), Bohning et al. (1975) found no effect of the fluorocarbons fluorotrichloromethane (FC-11) and dichlorodifluoromethane (FC-12) on tracheobronchial particle deposition and mucociliary clearance in donkeys. The animals were intermittently exposed to 1000-ppm concentrations over a period of about 13 months. The authors noted that broncopulmonary changes have, however, been observed in mice (Brody, Watanabe, and Aviado, 1974) and in humans (Brooks, Mintz, and Weiss, 1972; Sterling and Batten, 1969) at higher concentrations of the propellants. The conclusion is that the threshold limit value of 1000 ppm is quite reasonable.

Flowers, Hand, and Horan (1975) studied the concentrations of fluoroalkanes associated with cardiac conduction system toxicity. No deaths ensued in dogs exposed for 10 min to FC-11 at concentrations below 15%. At concentrations between 15% and 17%, eight animals survived and seven died. No animal survived a 10-min exposure to a concentration in excess of 21%. For FC-12, concentrations greater than 95% were necessary to produce death in 10 min, with severe oxygen deficit. The results for FC-11 are summarized in Figures 6.26 and 6.27. The narrow margin between

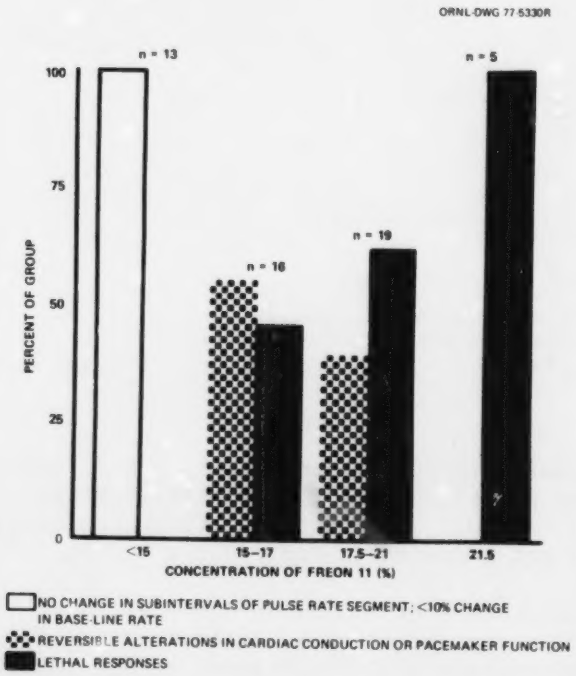

Figure 6.26. Effect of Freon 11 on cardiac conduction in dogs. Source: Adapted from Flowers, Hand, and Horan, 1975, Figure 8, p. 360. Reprinted by permission of the publisher.

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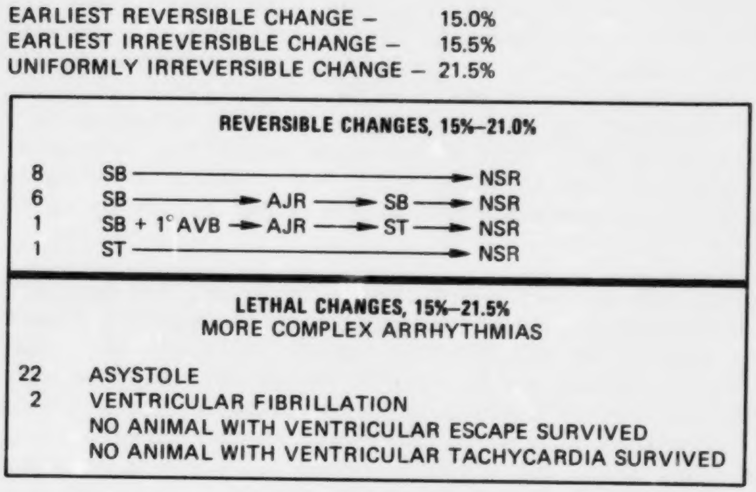
EARLIEST REVERSIBLE CHANGE – 15.0%
EARLIEST IRREVERSIBLE CHANGE – 15.5%
UNIFORMLY IRREVERSIBLE CHANGE – 21.5%
REVERSIBLE CHANGES, 15%–21.0%
8 SB → NSR
6 SB → AJR → SB → NSR
1 SB + 1° AVB → AJR → ST → NSR
1 ST → NSR
LETHAL CHANGES, 15%–21.5%
MORE COMPLEX ARRHYTHMIAS
22 ASYSTOLE
2 VENTRICULAR FIBRILLATION
NO ANIMAL WITH VENTRICULAR ESCAPE SURVIVED
NO ANIMAL WITH VENTRICULAR TACHYCARDIA SURVIVED

Figure 6.27. Freon 11 results: note narrow margin between concentration eliciting earliest detectable yet reversible change and concentration of the first lethal result. Source: Adapted from Flowers, Hand, and Horan, Figure 9, p. 360. Reprinted by permission of the publisher.

the concentration of the first lethal results should be noted. The study sounded a note of alarm because lethal concentrations of fluorocarbons can be reached by "sniffing" and indeed have been. The study also emphasized that the concentrations are orders of magnitude higher than those found in certain areas (use of fluorocarbon-propelled hair sprays in beauty salons was mentioned). Thus to uniformly produce death in the test animals, a continuous 10-min exposure to 21.5% FC-11 (215,000 ppm) was required. Peak concentrations in samples obtained over several months from a beauty salon did not exceed 310 ppm, which is well below the industrial threshold limit value of 1000 ppm, whereas simulated sniffing episode samples reached 350,000 and 400,000 ppm. Where use is fairly heavy, good ventilation is recommended.

Paulet and Lessard (1975) studied the action of FC-11 and FC-12 on smooth muscle. Both gases, bubbled into the muscle nutritive solution to give various concentrations, reduced the amplitude of spontaneous contractions of both isolated rat uterine muscle and isolated rabbit duodenum muscle. Base tonus of the rat muscle was not much affected by either gas; with rabbit muscle, FC-12 increased tonus while FC-11 decreased it. The effects of both compounds were shown to be musculotropic, not neurotropic, and to be reversible, even after prolonged inhibition. The two gases did not modify the action of acetylcholine, adrenalin, histamine, or oxytocin on the muscles.

Paulet, Roncin, Vidal, Toulouse, and Dassonville (1975) also reported on the effect of fluorocarbons on the general metabolism of rats, rabbits, and dogs. In single inhalation exposure experiments, only FC-11 produced metabolic reactions; for example, at 5% a slight hyperglycemia with lactacidemia was produced. In prolonged exposure experiments (inhalation 2 hr/day for five days), only FC-11 at 5% produced the following slight metabolic modifications: decrease in oxygen uptake, increase in glycemia and lactacidemia, slowing of hepatic glycogen secretion, increase in respiratory quotient, decrease in blood urea, and a small increase in free fatty acid. All of these changes could be explained by a slowing of cellular oxidation.

As studied by Paulet, Lanoë, Thos, Toulouse, and Dassonville (1975), FC-11 and FC-12, when inhaled in concentrations varying from 0% to 5% (FC-11) and from 0% to 50% (FC-12), rapidly diffused into the blood, cerebrospinal fluid, urine, and bile; a balanced condition is quickly established. This effect is similar to the results of Niazi and Chiou (1976). The rapid appearance (1 min) of the gases in the cerebrospinal fluid is noteworthy, explaining the often observed stimulating or depressing action of fluorocarbons on the central nervous system. Elimination takes place primarily through the lungs (98%) and is completed 20 to 50 min after fresh air is inhaled. Bile and urine constitute only a minor pathway of excretion.

6.4.3.6.2 Fluorine-containing anesthetics — Krantz and Rudo (1966) reviewed the history of the introduction of fluorinated anesthetics and their chemistry and use. Fluorine does not appear to increase the potency of a compound per se; it decreases flammability and augments the stability of associated halogens in the molecule. Saturated and unsaturated cyclic, branched, and straight-chain compounds containing fluorine and other halogens have been examined for use as anesthetics. While inhalation is the most common route of administration, injection of an emulsion has also been tried (Krantz et al., 1961). This allows use, with simplicity and accuracy of dosage, of compounds not otherwise usable because of low volatility. The injected agents are excreted by the lungs. Some fluorinated compounds can cause excitation as well as anesthesia, and this has ruled out their use. In the use of an anesthetic, effects on the heart, respiration, and the central nervous system must be considered, as well as toxic side effects such as hepatotoxicity and nephrotoxicity. Toxicity may arise from cell injury due to fatty infiltration and from effects of breakdown products if they exist. The toxicities of several of the fluorinated anesthetics used are considered in the following paragraphs.

Fluoromar or fluroxene (trifluoroethyl vinyl ether, $CF_3CH_2OCH{=}CH_2$) was the first fluorinated compound used for human anesthesia. It is useful for both light and deep anesthesia. Fluoromar is an excellent anesthetic because fluoride is not liberated by metabolic processes of the body, there is no hepatic impairment, and recovery is rapid with early return of reflexes. According to Morris (1963), it is the only halogenated drug not producing hepatic damage. However, it is somewhat lacking in potency compared with some of the other fluorinated anesthetics.

Halothane or fluothane ($CF_3CHBrCl$) undergoes some biotransformation in the body. It is more soluble in tissue compartments than in whole blood and causes some hepatotoxicity (Jones, Margolis, and Stephen, 1958); however, it causes less hepatotoxicity than chloroform. Because halothane causes some hepatotoxicity and decreases uterine tone, it is not recommended for patients with known liver or biliary tract disease or for obstetrical use. An azeotropic mixture of halothane and ether is a highly effective anesthetic.

The biotransformation of halothane was studied by Airaksinen, Rosenberg, and Tammisto (1970). Trifluoroacetic acid, the main metabolite found in the urine of man after halothane anesthesia, was probably formed through trifluoroethanol and trifluoroacetaldehyde. The results indicated that toxicity was chiefly caused by the blocking of essential thiol groups (-SH) by the aldehyde, or a compound derived from it, before oxidation to trifluoroacetic acid.

Methoxyflurane ($CH_3OCF_2CCl_2H$) has been extensively used as an anesthetic. Its relatively low vapor pressure allows some safety in administration. Its elimination is slow because of absorption by adipose tissues. Slow induction, slow emergence, and hypotension during anesthesia are disadvantages, but the quality of anesthesia is good. Some nephrotoxicity of methoxyflurane has been noted due mainly to fluoride released from the compound by hydrolysis (Hook, 1971; Mazze, Shue, and Jackson, 1971; Taves et al., 1970). Methoxydifluoroacetic acid is also produced. Cases of secondary hyperoxaluria following methoxyflurane anesthesia, with actual deposition of calcium oxalate crystals in the tubules if renal function was sufficiently compromised, were studied by Frascino, Vanamee, and Rosen (1970). There was enough concern that the National Academy of Sciences-National Research Council Committee on Anesthesia issued a "Statement Regarding the Role of Methoxyflurane in the Production of Renal Dysfunction" (National Academy of Sciences and National Research Council, 1971). It was considered that a chief mechanism of action was an effect of fluoride (from hydrolysis of the methoxyflurane) that rendered the distal renal tubules unresponsive to antidiuretic hormone. Figure 6.28 shows the serum levels of fluoride and "organic acid—labile fluoride" in patients at various times after methoxyflurane anesthesia. Since the two metabolites are not volatile, they are eliminated by the kidneys, not the lungs. It was suggested that levels might be lowered and the nephrotoxicity minimized by management of fluids and electrolytes.

Creasser et al. (1974) and Dahlgren (1977) reported on the effects of methoxyflurane used as an analgesic in obstetrics. In the study by Creasser et al. (1974), methoxyflurane exposure to 22 obstetric patients consisted of 65 to 240 min intermittently self-administered during labor and 5 to 70 min of awake analgesia during vaginal delivery. Some alteration in renal function was observed in the methoxyflurane group but not in the control group as indicated by an increase in blood urea nitrogen, serum uric acid, and serum creatinine following delivery. However, postpartum observation in the control group was limited to 24 hr and in no instance was any measured value in the control group significantly different from the measured values in the methoxyflurane group at 24 hr postpartum. The polyuric form of renal dysfunction is known to occur when

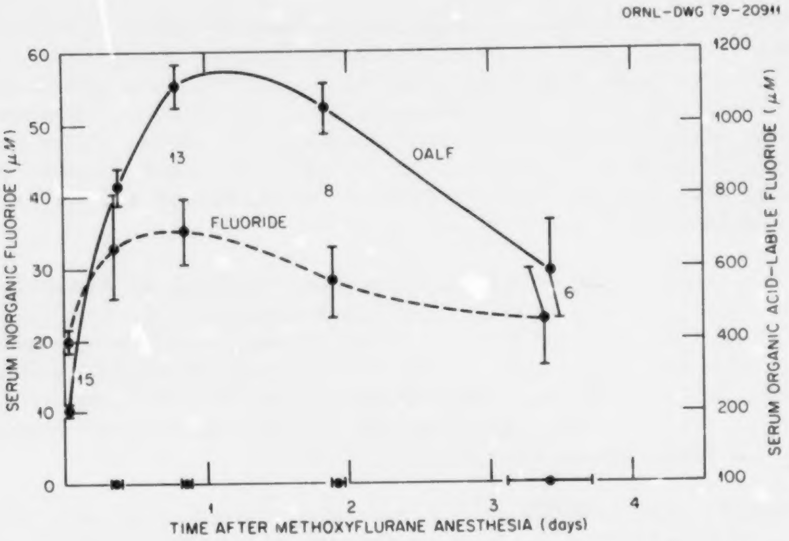

Figure 6.28. Serum fluoride and "organic acid-labile fluoride" (OALF) concentrations of all samples taken from 15 patients at various times after the end of anesthesia. The numbers are numbers of samples included in the means; bars are standard errors of the means. Source: Adapted from Fry, Taves, and Merin, 1973, Figure 1, p. 41. Reprinted by permission of the publisher.

serum inorganic fluoride concentrations reach 80 to 175 micromoles per liter, but it was not observed in the patients studied by Creasser and co-workers where the mean peak serum inorganic fluoride level was only 21.9 micromoles per liter. Although methoxyflurane analgesia during labor and vaginal delivery provided acceptable patient analgesia without fetal depression at birth, the presence of increased methoxyflurane metabolites (inorganic fluoride and oxalic acid) which might cause some alteration in renal tests suggested to Creasser et al. (1974) that there was a need to control the total dose of methoxyflurane administered to the mother.

Dahlgren (1977) studied the renal and hepatic effects of the administration of a mixture of nitrous oxide and methoxyflurane as an analgesic to women in labor; comparison was made to women in labor who were administered only nitrous oxide. In an earlier study Dahlgren observed that when using an analgesic mixture of thiopentone and methoxyflurane, thiopentone and halothane, or thiopentone and pethidine a transient disturbance of renal and hepatic function was associated with methoxyflurane (Dahlgren and Goodrich, 1976, as cited in Dahlgren, 1977). Dahlgren (1977) designed a study to test if renal and hepatic disturbances were associated with methoxyflurane and nitrous oxide analgesia and if signs of distal tubular dysfunction could be detected in neonates. The results of his studies with 75 women who received only nitrous oxide analgesia and with 126 women who received the methoxyflurane and nitrous oxide mixture (29 received a dose greater than or equal to 10 ml and 75 received a dose less than or equal to 5 ml) indicated that hepatic and renal functions were affected in the groups exposed to the nitrous oxide/methoxyflurane mixture (especially the high dose methoxyflurane group). This was indicated by statistically significant increases in serum sodium, serum creatinine, blood urea, serum uric acid, glutamic oxalacetic transaminase, and glutamic pyruvic transaminase. The neonatal capillary concentrations of uric acid also showed dose-dependent increases in response to methoxyflurane. Dahlgren stated that: (1) the significant differences between the nitrous oxide group and the nitrous oxide/high dose methoxyflurane group in serum sodium, serum creatinine, blood urea, and uric acid concentrations were in agreement with the results of other researchers and (2) as in the study by Robertson and Hamilton (1973) the major influence of the methoxyflurane anaesthesia was on serum uric acid concentrations. Robertson and Hamilton (1973, as cited in Dahlgren, 1977) suggested that the increase in serum uric acid concentrations was a toxic effect of the fluoride ion on the ability of the distal tubules to secrete uric acid.

Enflurane (CF_2HOCF_2CClFH) was developed to replace halothane and, for some uses, methoxyflurane. It undergoes biotransformation, but to a lesser degree than methoxyflurane. Thus Barr et al. (1974) found that it took 6 to 10 hr of 2.5% enflurane anesthesia to develop vasopressin-resistant polyuric renal dysfunction in rats similar to that resulting from 1.5 hr of 0.25% methoxyflurane. Peak serum inorganic fluoride levels were similar for both compounds at about 1.14 ppm; however, the level peaked earlier and returned to control values sooner after enflurane than after methoxyflurane anesthesia.

Figure 6.29 shows serum inorganic fluoride levels in 102 patients following enflurane anesthesia. The 102 patients were divided into three groups using the following criteria: group one patients were without pertinent medical or anesthetic history, group two patients had received one previous enflurane anesthetic, and group three patients had all taken barbiturates before enflurane anesthesia. For most individuals, the peak fluoride levels in all three groups were about the same; however, with methoxyflurane, fluoride levels might continue to rise or remain elevated for days and with enflurane the fluoride level generally declines within 24 hr.

In spite of possible nephrotoxicity, methoxyflurane continues to be used for obstetrical patients because of its valuable analgesic and anesthetic properties. Figure 6.30 shows plasma fluoride levels in mother and child following use of methoxyflurane for delivery only, labor and delivery, and delivery by cesarian section (mean exposure durations of 19.7 ± 5.9, 49.1 ± 23.9, and 39.6 ± 14.2 min respectively). The values in all cases were considered tolerable, and no nephrotoxicity was noted. Fry and Taves (1974) concluded that exposure of obstetrical patients to methoxyflurane for as long as 30 min was probably safe. For general anesthetic use of methoxyflurane, Cousins and Mazze (1973) gave a number of cogent recommendations as to dose, use of adjuvants, and type of surgical intervention with exposure to be limited to 2 hr at the lowest feasible concentration. They found the threshold inorganic fluoride level for biochemical renal impairment to be about 50 micromoles per liter of plasma

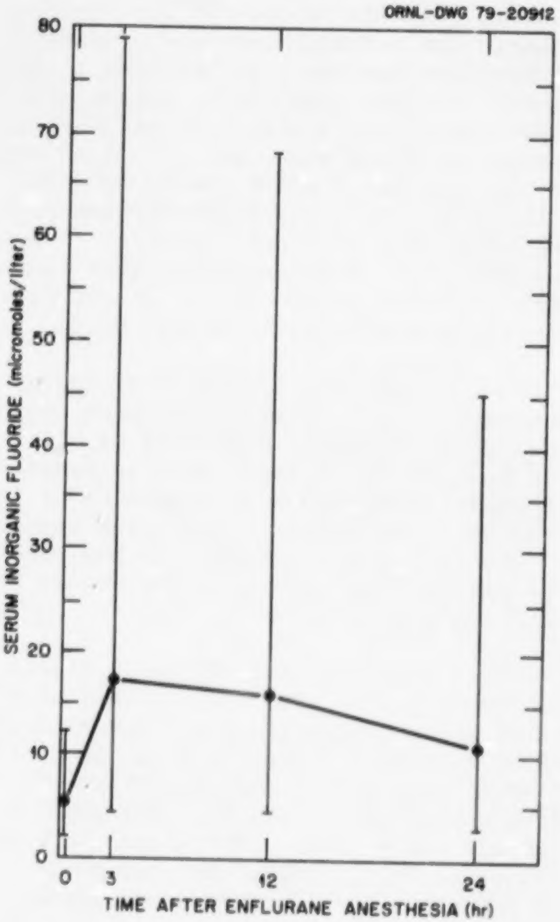

Figure 6.29. Mean serum inorganic fluoride levels for all three patient groups combined plotted against time in hours. Brackets indicate range of values. Source: Adapted from Maduska, 1974, Figure 1, p. 352. Reprinted by permission of the publisher.

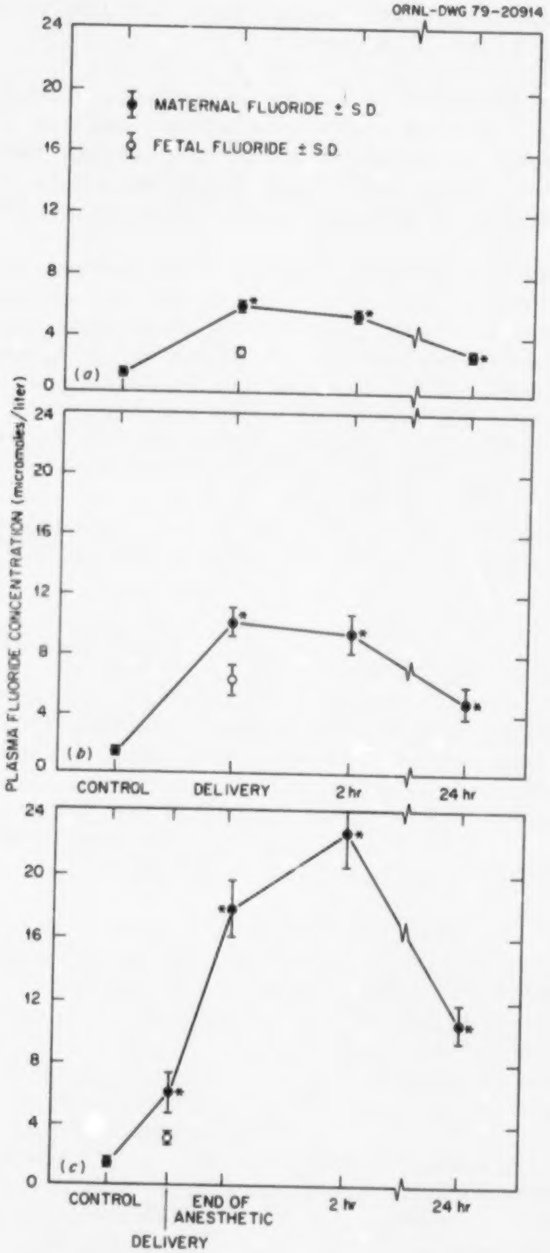

Figure 6.30. Plasma fluoride levels following methoxyflurane analgesia for (*a*) delivery only, (*b*) for labor and delivery, and (*c*) for caesarean section (* indicates significant change from control). Source: Adapted from Palahniuk and Cumming, Figures 1-3, pp. 294-295. Reprinted by permission of the publisher.

(0.95 ppm), with clinical high output renal failure becoming evident at 100 micromoles per liter (1.9 ppm).

Impurities in anesthetics may be hazardous. Torkelson et al. (1971) studied the question of the acute inhalation toxicity of actual and potential trace substances in methoxyflurane by exposing rats to the vapors of nine such compounds. None were found toxic enough to present a significant hazard in the use of methoxyflurane as an anesthetic.

The fluorinated anesthetics show a lower mortality rate than the anesthetics they replace and are a useful advance as anesthetics. What hazards they do present (e.g., hepatotoxicity, renal toxicity, and cardiac and circulatory sensitization) can be minimized by proper management and selection of the best anesthetic for a particular use. It is likely that research will improve the situation even further. Two questions, however, need to be considered. One concerns the possible embryopathic, teratogenic effects of anesthetics given during pregnancy and the effect on reproduction function. The other questions the effects on the offspring of individuals who constantly come in contact with halogenated anesthetics (e.g., anesthetists and operating room personnel). These questions are discussed in Section 6.4.4.

6.4.3.6.3 Fluorinated compounds as blood extenders and other medical uses — Gollan and Clark (1966) pioneered the use of oxygenated liquid fluorochemicals for organ perfusion and carried animals through alternate episodes of breathing liquid fluorocarbon and then air (Clark and Gollan, 1966). Sloviter and Kamimoto (1967) introduced the use of emulsions of fluorochemicals in water-protein solution in order that salts and metabolites might be carried as well as gases. Geyer, Monroe, and Taylor (1968) and Clark, Kaplan, and Becattini (1970) succeeded in keeping animals alive whose blood was totally replaced with such emulsions.

Fluorinated compounds are considered to be inert in the sense that no degradation occurs. They do, however, interact under some conditions with cell substances and cell systems. For instance: in influencing the metabolism of steroids (Höller and Breuer, 1975); causing possible structural changes in amino acids (Brown and Hardison, 1975); and interacting with platelets and blood-clotting factors resulting in intravascular clotting causing a marked increase in pulmonary artery pressure, dilation of the right side of the heart, and terminating in fatal hypoxia (Sloviter, 1975). Okamoto, Yamanouchi, and Yokoyama (1975) studied the elimination of fluoro compounds because their long retention prohibits their use in some situations. There has been no clinical use yet of fluorochemicals or other synthetic substances as erythrocyte substitutes (Sloviter, 1975), but such use is not inconceivable. Use in extracorporeal circulation, membrane oxygenation systems, and organ isolation experiments is also possible. The field is very active. According to Clark et al. (1975) the future use of highly fluorinated compounds in biology and medicine lies in the great versatility that can result by changing their chemical structure. In fact, numerous types of fluorinated compounds have already been used; for example, cyclic ethers and other ring compounds and straight- and branched-chain structures. The use of fluoro compounds in a liquid membrane configuration for blood oxygenation, to avoid the formation of actual blood-gas interfaces that might cause damaging cellular or protein alterations, is a relatively new development (Maugh, 1976).

Fluorinated compounds containing a heavier halogen in the molecule have also been used as radio-opaque media. Examples are perfluorooctyl bromide and perfluorohexyl bromide (Long et al., 1972) and perfluorooctyl iodide and perfluorodecyl iodide (Clark et al., 1975). An interesting application of a fluorinated compound was the trial of octafluorocyclobutane and other gases for vitreous replacement (Vygantas et al., 1973), especially in treatment of retinal detachment. A gas was sought that would be slowly reabsorbed. The results were encouraging, and no toxic effects were noted.

6.4.3.6.4 Fluorinated fire-extinguishing agents — Tables 6.18 and 6.19 show the lethal concentrations and toxicities of vapors from various agents used in fire fighting. Bromotrifluoromethane is seen to be in a higher UL class than even carbon dioxide. Table 6.20 shows the minimal concentrations of various halogenated fire-extinguishing agents required to produce characteristic reactions in rats, and Table 6.21 compares the toxicity of several agents under various fire situations.

The best agent tried, bromotrifluoromethane (CF_3Br), has been used in portable extinguishers since 1959 by the U.S. Army and in specialized situations such as protection against in-flight engine fires in commercial aircraft. It is in some canister-type extinguishers that can be bought by the general public and is used in preference to carbon dioxide where space is at a premium; for example, to protect specialized equipment such as computers. Its advantages are discussed by Mosbacher (1976). The toxicity of pyrolysis products of fire-extinguishing agents in confined

TABLE 6.18. LETHAL CONCENTRATIONS OF SEVERAL FIRE-EXTINGUISHING AGENTS

Compound	Species	Time of exposure (min)	Lethal concentration (%)[a]
CCl_4	Rat	10	2.8
	Guinea pig	10	2.0
CH_2BrCl	Rat	15	2.9-3.2
	Mouse	15	2.7-2.9
CBr_2F_2	Rat	15	5.5
$CBrClF_2$	Rat	15	32
$CBrF_3$	Rat	15	83

[a] Vapor mixed with air in all exposures.

Source: Adapted from Clayton, 1966, Table 2, p. 463. Data collected from several sources. Reprinted by permission of the publisher.

TABLE 6.19. TOXICITIES OF VARIOUS GASES ENCOUNTERED IN FIRE FIGHTING

Compound	Formula	UL[a] class	ALC[b] (% vol)	ALC decomposition product (% vol)
Bromotrifluoromethane	$CBrF_3$	6	83.2	1.4 2.0
Carbon dioxide	CO_2	5a	65.7	
Difluorodibromomethane	CBr_2F_2	4	5.5	0.2
Bromochloromethane	CH_2BrCl	3	6.52	0.4
Carbon tetrachloride	CCl_4	3	2.86	0.032
Carbon monoxide	CO	2	1.5	
Methyl bromide	CH_3Br	2	0.59	1.6

[a] Underwriters' Laboratories.

[b] Approximate lethal concentration in 15-min rat exposure; vapors mixed with air in all exposures.

Source: Adapted from Clayton, 1966, Table 4, p. 464. Data collected from several sources. Reprinted by permission of the publisher.

TABLE 6.20. MINIMAL CONCENTRATIONS OF HALOGENATED FIRE-EXTINGUISHING AGENTS REQUIRED TO PRODUCE CHARACTERISTIC REACTIONS IN RATS[a] (ppm)

Reaction	Chlorobromomethane, CH_2BrCl	Trifluorobromomethane, $CBrF_3$	Difluorodibromomethane, CBr_2F_2	Tetrafluorodibromoethane, $CBrF_2 \cdot CBrF_3$	Carbon tetrachloride, CCl_4
Incoordination	4,750 (26 min)			3,400	9,000 (14 min)
Drowsiness	4,750 (26 min)	500,000 (26 min)	10,000 (14 min)		9,000 (20 min)
Limb twitching and slight body tremors			15,000 (10 min)	13,500 (14 min)	15,500 (12 min)
Convulsions and acute body tremors			30,000 (11-13 min)	27,000 (9 min)	
Deep narcosis	19,000 (17 min)				18,000 (9-11 min)
Delayed death (about 36 hr)					18,000
Liver damage					18,000
Death during exposure	39,000 (30 min)				27,000

[a] Time of onset of reactions in parentheses.

Source: Adapted from Clayton, 1966, Table 9, p. 467. Data collected from several sources. Reprinted by permission of the publisher.

TABLE 6.21. TOXICITIES OF FIRE-EXTINGUISHING AGENTS

Condition	Toxicity
Rapid fire extinguishing[a]	$CBr_2F_2>CH_2BrCl>CBrF_3$
Slow fire extinguishing[a]	$CBr_2F_2>CH_2BrCl>CBrF_3$ CCl_4
Evolution of carbonyl halides (slow extinguishment)	$CCl_4>CH_2BrCl>CBrF_3$

[a] Gasoline fire.

Source: Adapted from Clayton, 1966, Table 10, p. 467. Data collected from several sources. Reprinted by permission of the publisher.

situations is of some concern. Table 6.22 shows the mortality and response of several mammalian species to pyrolyzed CF_3Br. The total gas concentration (decomposed and undecomposed) was 10,000 ppm. The experimental conditions were more severe than would usually be encountered by humans in fire-fighting situations.

Bromotrifluoromethane shares with some other compounds, such as carbon tetrachloride and gasoline, the capability of causing the heart muscle to react abnormally to elevated adrenaline levels. This might occur in a stress situation such as a fire. The resulting cardiac arrhythmia can cause death. Tests by Haskell Laboratory (1971) showed such cardiac sensitization in dogs under adrenaline challenge starting at about 7.5 vol % bromotrifluoromethane. In humans, central nervous system effects (e.g., lightheadedness and impaired reaction times) were observed when levels of 7% were breathed for 4 min, but no cardiac arrhythmias occurred at levels

TABLE 6.22. MORTALITY AND RESPONSE OF SEVERAL MAMMALIAN SPECIES TO PYROLYZED BROMOTRIFLUOROMETHANE (CF_3Br)[a]

CF_3Br concentration[b] (% vol)	Mortality (%)	Animal response	Pathology	Species	Number of animals
50	100	Initial agitation, somnolence, effect on equilibrium, dyspnea, prostration	Pulmonary edema	Mice Rats Guinea pigs	10 10 5
2.5	100	Slight agitation, dyspnea	Pulmonary edema	Mice Rats Guinea pigs	10 10 5
1.0	100	Few reactions, slight dyspnea, death in 48 hr	Pulmonary edema	Mice Rats Guinea pigs	10 10 5
0.75	90 20	No reactions	Pulmonary edema in animals succumbing	Mice Rats	10 10
0.50	50 0	Death occurred in mice within 3 days after exposure	Pulmonary edema in mice	Mice Rats	10 10

[a] Exposure: Pyrolysis temperature 1000°C, 30 min.
[b] Based on air mixed with decomposed and undecomposed $CBrF_3$.

Source: Adapted from Clayton, 1966, Table 7. Data collected from several sources. Reprinted by permission of the publisher.

up to 10%. Rhoden and Gabriel (1972) studied the effect of CF_3Br inhalation on myocardial glycolysis in rats and showed respiratory depression, hypoxia, and mobilization and intracellular accumulation of free fatty acids. The Haskell report considered that in spite of possible deleterious effects, CF_3Br is the safest gaseous extinguishing agent available. Low concentrations are required to put out fires, and it acts quickly; its toxic effects are less than those caused by products of the fire. Its chief pyrolysis product, hydrogen fluoride, is toxic but gives warning by its extremely irritating nature.

The workroom threshold limit value for CF_3Br is 1000 ppm (American Council on Governmental Industrial Hygienists, 1971), but this is an arbitrarily set "industrial housekeeping" level (Section 6.4.3.6.1). Scholz and Weigand (1964) found no behavior changes in animals inhaling CF_3Br at concentrations as high as 60 vol % (with oxygen) for a single 2-hr period and no damage to health in workers engaged in the manufacture of CF_3Br. Rhoden and Gabriel (1972) drew a parallel between deaths caused by aerosol-sniffing incidents and cardiac effects from CF_3Br and were pessimistic as to the desirability of its use in closed environments.

6.4.4 Teratogenesis, Mutagenesis, and Carcinogenesis — On the basis of the chemistry and of the enzyme, hormone, and other effects discussed in preceding sections, inorganic fluoride under normal conditions would not be expected to be teratogenic, mutagenic, or carcinogenic. In contrast, some organic fluorinated compounds are indeed used experimentally as mutagens, and fluorinated compounds are also used as carcinogens. This is not of environmental concern. Other fluorinated compounds such as 5-fluorouracil are used as cancer-control agents (Heidelberger, 1972). On the basis of differences in metabolism between cancerous and normal cells, inorganic fluoride has been tried as one of a number of glycolysis inhibitors in cancer treatment (Black and Kleiner, 1947; Black, Kleiner, and Bolker, 1947, 1949). While the results were initially encouraging, at least with some kinds of cancer, adaptation to the inhibitor(s) resulted in no great long-term benefit, and the approach has been discarded.

Regarding inorganic fluoride effects on the general population, controlled health studies have shown no greater incidence of birth defects or of cancer in areas where water was fluoridated or where the level was naturally high than in low-fluoride areas. The normality of the size and development of children in the American Southwest, where drinking water supplies contain up to 5 ppm fluoride, was never questioned, nor were any differences in developmental rates found between children in Newburgh, New York, where water was fluoridated to a concentration of 1 ppm, and children in Kingston, New York, the control city with a community water supply containing 0.06 ppm (National Academy of Sciences, 1971, pp. 198-199). Schlesinger (1970) gave a detailed analysis of the Kingston-Newburgh study. The stillbirths and maternal and infant mortality rates in the two cities were compared for the five-year period prior to the start of fluoridation and during the ten years of the study. The long-term downward trends were similar in the two cities, and no changes could be detected in the trend in Newburgh after fluoridation. The death rates from cancer and cardiovascular diseases were also examined in the two cities and did not change, relative to each other, prior to or during the period of the study.

Archer and Lundin (1967) compared lung cancer data from five groups of miners exposed to a wide range of airborne radioactive particles (decay products of radon). Included was a group of Canadian fluorspar miners. The emphasis was on radiation damage, and no synergism with fluoride was suggested. Kanisawa and Schroeder (1969) fed mice 10 ppm fluoride for life and found neither suppression nor enhancement of tumors. Gorban, Karpilovskaya, and Rubenchik (1974) have shown, however, that sodium fluoride added to the diet at 1.5 mg/kg daily for four months inhibited the development of hepatomas induced in rats by *p*-dimethylaminoazobenzene and also prevented increase in the level of glycogen in the liver.

Cecilioni (1972, 1974) reported that the mean cancer death rate in the highly industrialized city of Hamilton, Ontario, was significantly higher than in the less industrialized city of Ottawa, Ontario, during 1966 to 1971. Cecilioni attempted to correlate the cancer incidence and mortality with industrial pollution by steel mills; fluoride is one of several pollutants emitted. He presented evidence (e.g., hospital data on respiratory diseases) that showed a good correlation between industrial pollution and cancer incidence, but no one pollutant could be conclusively implicated.

Several papers have been written that discuss the potential mutagenicity of inorganic fluoride compounds to laboratory animals and human subjects. Guleva, Plotko, and Gatiyatullina (1972) subjected rats to inhalation of cryolite (AlF_6Na_3) and its mixture with hydrogen fluoride. While there were no changes in the mitotic activity of test corneal epithelial cells as compared with the control ones, the rats presented a high percentage of cells with chromosome aberrations in the bone marrow. Jagiello and Lin (1974) explored the potentiality of fluoride as a mutagen in mammalian eggs. In vitro experiments with mouse, sheep, and cow oocytes incubated in solutions containing up to 0.4 mg of sodium fluoride per milliliter (181 ppm fluoride) disclosed a low incidence of anaphase lags, suppression of polar body I, and fragmentation with rearrangement. In vivo injection experiments showed only a minor effect on oocyte meiotic maturation. Species variations in sensitivity suggested, however, an assessment of abnormal progeny from cows and ewes for chromosome abnormalities in high-fluoride contaminated areas.

The mutagenic action of hydrogen fluoride, cryolite, and mixtures of both on the chromosomes of cells from albino rats and white mice (inhalation exposure of 6 hr/day, six days per week for two or five months), and of the mutagenic action of sodium fluoride on the culture cells of human leukocytes was studied by Voroshilin et al. (1973). Male albino rats exposed for five months showed an increase in the frequency of cells with chromosomal damage in the bone marrow when the cryolite concentration was 3.0 mg/m^3 and when a mixture of cryolite and hydrogen fluoride contained 0.5 mg/m^3 and 0.25 mg/m^3 respectively. A two-month exposure of white mice, however, resulted in no increased frequency of translocations in testes cells when the hydrogen fluoride exposure concentration was 0.1 mg/m^3 and the mixture concentration was 0.25 mg/m^3 of cryolite and 0.05 mg/m^3 of hydrogen fluoride. Similarly, no increase in the frequency of chromosomal aberrations was observed in the cell culture of human leukocytes in the G_0 stage at sodium fluoride concentrations of 1×10^{-3} and 3×10^{-3} *M*; at higher concentrations sodium fluoride was cytostatic.

Mohamed and Chandler (1976) divided 72 adult male highly inbred mice into nine equal groups, with three groups being controls and the remaining six groups exposed to 200, 100, 50, 10, 5, and 1 ppm sodium fluoride, respectively, through the drinking water; mice were maintained on a low fluoride diet. Four of the eight mice in each group were exposed for three weeks and the other half for six weeks. Cytological examinations on bone marrow cells and on spermatogenesis indicated the presence of fragments, bridges, and other chromosomal abnormalities. The physiological effect of sodium fluoride causing stickiness of chromosomes and/or the occurrence of chromatid breakage followed by reunion to form structural changes was suggested as the cause of these abnormalities. Mohamed and Chandler stated that the analyses of their findings indicated that sodium fluoride was a mutagenic agent with cumulative effects.

Brown et al. (1978) noted the results of Mohamed and Chandler (1976) and conducted experiments to examine the rates of chromosome aberration in bone marrow cells and in cells of testes from mice raised under various fluoride intakes. In one experiment, Swiss mice that had been administered 50 ppm fluoride in the drinking water showed no difference in aberration rate in the testis or marrow cells compared to a control group that had received distilled water; both control and experimental groups were fed low fluoride diets (0.5 ppm). In contrast to the results of Mohamed and Chandler (1976), this experiment showed that chromosome aberrations were not related to continuous fluoride intake. A second experiment by Brown et al. (1978) in which adult male BALB/c mice were exposed to fluoride concentrations of 0, 1, 5, 10, 50, or 100 ppm in the drinking water for six weeks also showed no dose-related differences in chromosome aberration rates of either marrow or testis cells. In addition, Brown and co-workers tested fluoride for mutagenic activity in a microbial assay using both *Salmonella* and *Saccharomyces* cells and found no evidence of mutagenicity.

Fluoride is known to have an antimutagenic action in some circumstances (Obe and Slacik-Erben, 1973). In studies of cultures of various types and species of cells, including human ones, sodium fluoride alone had no effect. However, it opposed the action of various polyfunctional alkylating agents, apparently suppressing one or several of the events that normally lead to breakage of chromosomes following attack of the alkylating agents.

At high doses fluoride apparently is able to induce certain malformations in laboratory animals. Fleming and Greenfield (1954) administered fluoride as sodium fluoride or calcium fluoride to female adult Webster mice during gestation in cumulative doses as high as 600 to 700 μg CaF_2 and 1000 to 1200 μg of sodium fluoride. Neonatal mice showed changes in the structure of the jaws and teeth, retarded calcification of bone in the jaws, alteration of the cell structure of the ameloblast, and retardation of the enamel matrix maturation. In another study, female Wistar albino rats were given oral doses of sodium fluoride (2, 5, or 10 mg/g daily) for 4 days from the 8th to the 11th day (Group A) or from the 11th to the 14th day (Group B) of gestation (Goto, 1968). Fetuses observed at term showed no external malformations; however, skeletal observations showed that Group B fetuses had retarded ossification in the occiput, sternbrae, metacarpal bones, metatarsal bones, and caudal vertebrae, and in Group A some fetuses had various skeletal abnormalities. The delivered young showed that three weeks after birth the retarded ossification was restored to the normal condition. In addition, the growth of groups' A and B sucklings was almost the same as the control sucklings. D'Angelo and Esposito (1965) found that doses of 25 mg/kg (almost the minimal lethal dose) given parenterally or orally to pregnant rats (D'Angelo and Esposito, 1965) produced fetal abnormalities of liver, kidneys, skull, jawbones, and teeth.

Five articles are listed under the heading "Fluorine" in Shepard's *Catalog of Teratogenic Agents* (Shepard, 1973). One is the article by Fleming and Greenfield already discussed. A report by Schour and Smith (1940) on the experimental and histologic analysis of mottled teeth and a report by Smith and Smith (1935) on the occurrence of mottled enamel in the temporary teeth are included. There is also a study by Spratt (1950), using explanted chick embryos (in vitro studies), on the metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors. Fluoride had little effect on the developing central nervous system but considerable effect on the heart. Iodoacetate acted oppositely. Spratt's explanation of this was the relatively greater dependence of the heart tissue on glycolysis (fluoride's action on enolase would block this) as contrasted with the dependence of brain tissue on respiration. Duffey and Ebert (1957) extended Spratt's work, confirmed the differential effect of fluoride, and carried the explanation further to include the whole flow of electrons to oxygen, the two tissues having different makeups with respect to this.

Data presented by Rapaport (1956) from births in 14 U.S. cities in four states showed an apparent increase in mongolism with increase in the fluoride content of the water. This conclusion was criticized on sampling and statistical groups by Russell (private communication, 1962, as cited in Hodge and Smith, 1965) and shown to be spurious. A study by Berry (1958) in England and a study by Needleman, Pueschel, and Rothman (1974) in Massachusetts also showed no relation between the fluoride content of the water supply and the incidence of mongolism.

One final study also demonstrated that there is no association between water fluoridation and congenital malformations (Erickson et al., 1976). The incidence of selected malformations (including Down's syndrome) in areas with fluoride supplementation of public water supplies was compared with the incidence of malformations in areas where the water supply was deficient in fluoride; no substantial or significant differences were found.

The possibility of birth defects and reproductive malfunction arising from chronic exposure to halogenated anesthetics was mentioned in Section 6.4.3.6.2. The question of teratogenicity of anesthesia was reviewed by Smith (1974). Danger to mother and fetus from the anesthesia and anesthetics in operations during pregnancy has long been recognized; now concern is arising regarding the danger to anesthetists and other operating room personnel from chronic exposure to inhalation anesthetics. Surveys (e.g., questionnaires and examination of records) have shown an apparent greater incidence of miscarriages, infertility, and birth defects in the offspring of persons so exposed. The surveys have not yet been properly statistically analyzed, and, as Smith points out, other factors may also be implicated. Nonetheless, the available evidence suggests a need for caution. Concern is such that the FDA proposes to study the question further (Scott, 1976). If a danger is clearly shown, steps such as more rigorous control of ventilation and of release of anesthetics in the operating room (Whitcher et al., 1975), restriction in use of some anesthetics, different anesthetic mixtures, and greater use of injection of anesthetics as opposed to inhalation in order to minimize release of the agent may have to be instituted.

SECTION 6

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SECTION 7

ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

7.1 SUMMARY

Fluorine is widely used in industry, primarily as the mineral fluorspar, a metallurgical fluxing agent needed in the manufacture of steel, and as hydrofluoric acid, the most important manufactured compound of fluorine.

Since World War II, consumption of fluorides has increased greatly, and the U.S. demand by the year 2000 is expected to be 1,900,000 to 2,500,000 metric tons (basis: contained fluorine). It is unlikely, however, that this demand can be met domestically unless a practical method can be developed for recovering fluoride from wastes produced during phosphate rock processing. If economical recovery is not feasible, future consumption will depend on imports from Mexico, Spain, and Italy.

Major industrial emissions of fluoride to the atmosphere include coal combustion, phosphorus and phosphate fertilizer manufacturing, steel and aluminum production, and ceramic products manufacture. Natural emissions result from weathering of rocks and minerals and volcanic activities.

Fluorine is ubiquitous in the earth's crust. Igneous rocks average 625 ppm fluoride, sandstones about 270 ppm, shales about 740 ppm, and limestones about 330 ppm. Normal mineral soils average 200 to 300 ppm, sandy soils containing less than clays. Soils high in phosphorus (either naturally or as a result of fertilization) are enriched with fluoride; so are soils near certain industrial activities.

Fluoride tends to persist in soils with high calcium or aluminum content. Sandy, acidic soils favor development of soluble forms that may leach or be taken up by plants. The plant depletion time for fluoride in soil is estimated at 10,000 years.

Most natural waters contain fluoride, but concentrations in uncontaminated surface and groundwaters usually do not exceed 0.3 ppm and 10 ppm respectively. Nearly all U.S. public water supply systems meet U.S. Public Health Service standards for fluorides. Seawater of normal salinity averages 1.4 to 1.5 ppm fluoride. Highly saline or acid springs in active volcanic areas may contain up to 5000 ppm. Fluorides are removed from most waters by chemical precipitation; sediments contain an average of 730 ppm fluoride.

Fluorine may exist in the atmosphere as soluble gaseous fluorides or soluble and insoluble dusts; it only rarely occurs in elemental form. In uncontaminated rural areas, fluoride is present only in fractions of a part per billion (ppb). Urban U.S. air usually contains less than 0.06 ppb, but average atmospheric fluoride concentrations may exceed 1.25 ppb near some industries. Fluorides in the air return to earth as dusts or in condensation or precipitation.

Fluorides are removed from industrial wastes by precipitation or sorption methods. Lime is frequently used to precipitate fluoride. Sorptive methods involve passing fluoride wastes through scrubber towers or columns packed with a suitable substrate such as alumina or charcoal.

7.2 PRODUCTION AND USAGE

Fluoride is widely used in industry and is expected to be in greater demand in the future. The major industrial source of fluorides is fluorspar (fluorite), although cryolite, fluorapatite, and topaz may occasionally be used (Worl, Van Alstine, and Shawe, 1973). In 1977 the United States consumed nearly 1.2 million metric tons of fluorspar, 85% of which was imported (*Chemical and Engineering News*, 1978). Iron and steel producers and the chemical industry are the chief consumers of fluorspar. Iron and steel manufacturers use fluorspar chiefly in mineral form, but most chemical applications require hydrofluoric acid which is obtained by treating the mineral with sulfuric acid. In 1974, iron, steel, and electrometallurgical fluxing accounted for 44% of the total U.S. consumption of fluorspar, with the chemical industry a close second at 32.5%. Nonferrous metal production, chiefly aluminum and magnesium, accounted for 22%. The balance was distributed between ceramics and glass manufacture (1.3%) and other miscellaneous uses (0.2%) (Wood, 1975). Uses of important fluoride-containing compounds are given in Table 7.1, and supply-demand relationships are illustrated in Figure 7.1.

World consumption of fluoride has greatly increased since World War II. In spite of increased demand, however, domestic production has decreased. In 1970, the United States produced only 20% of the fluorspar it consumed, importing the balance from Mexico, Spain, and Italy (Worl, Van Alstine, and Shawe, 1973). Expected U.S. demand for fluoride in the year 2000 is 1,900,000 to 2,500,000 metric tons (2,100,000 to 2,700,000 short tons) (Table 7.2). The rest of the world is expected to consume 5,000,000 to 6,000,000 metric tons (5,500,000 to 6,600,000 short tons) (MacMillan, 1970). U.S. demand can be met domestically if an economical method for fluoride recovery from wastes produced during phosphate rock processing is devised. Otherwise, the presently known U.S. resources of fluoride in fluorspar will be depleted in 25 years.

In the United States, most fluorspar is mined from deposits located in Illinois and Kentucky. Important production also comes from Colorado, Utah, Idaho, New Mexico, Montana, and Nevada (Largent, 1961). U.S. fluoride resources (as fluorspar) are estimated at 4,900,000 metric tons (5,400,000 short tons). As demand for fluoride increases, it is thought that the rate of discovery of fluorspar reserves and the recovery of fluoride from other sources will also increase (MacMillan, 1970).

7.3 DISTRIBUTION OF FLUORIDE IN THE ENVIRONMENT

7.3.1 Sources of Pollution

7.3.1.1 Industrial Sources — Fluoride is emitted to the environment from a variety of sources (Table 7.3). Most emissions result from processing

TABLE 7.1. USES OF SELECTED FLUORIDE COMPOUNDS

Compound	Formula	Use
Aluminum trifluoride	AlF_3	Component of electrolyte melt in the manufacture and refining of aluminum; in ceramics and glass; glazes and enamels; welding rods
Ammonium bifluoride	NH_4HF_2	Frosting glass, boiler scale removal, wood treatment, stain removal
Antimony trifluoride	SbF_3	Manufacture of Freon-type compounds
Antimony pentafluoride	SbF_5	Oxidizing and fluorinating agent
Calcium fluoride	CaF_2	Raw material for production of fluorine compounds, production of plastics, steel manufacture, glass manufacture, cement, abrasives, welding rods
Cryolite	Na_3AlF_6	Aluminum manufacture, glass manufacture, enamels, grinding wheels, insecticides
Cupric fluoride	CuF_2	Construction of cathodes in nonaqueous galvanic cells, flux in casting gray iron
Perchloryl fluoride	ClO_3F	Oxidizer for rocket engines, cutting and welding metals, explosives
Hydrogen fluoride	HF	Etching and polishing glass, aerosol propellants, foaming agents, cleaning fluids, plastics, petroleum, pickling metals, dyes, pharmaceuticals, stain removers, catalyst for high-octane aviation fluid
Lead fluoride	PbF_2	Fluorinating agent, raw material for lead tetrafluoride, underwater paints, special glasses
Lithium fluoride	LiF	Aluminum welding fluxes, zirconium and titanium brazing fluxes, soft enamels and glazes, and depolarizer in fluorine manufacture
Magnesium fluoride	MgF_2	Flux in magnesium metallurgy, porcelain and pottery, phosphor for cathode-ray screens, coating agent for titanium pigments, antireflecting agent
Nitrogen trifluoride	NF_3	Rocket fuel oxidants, hydrogen-nitrogen trioxide torches
Oxygen difluoride	OF_2	High-energy rocket propellant
Potassium fluoride	KF	Fire extinguisher for alkali metal fires, preparation of silver-soldering fluxes
Potassium bifluoride	KHF_2	Electrolyte for preparing elemental fluorine, silver-solder flux, frosting of glass
Silicon tetrafluoride	SiF_4	Manufacture of fluorosilicic acid, preparation of concrete, oil production
Fluorosilicic acid	H_2SiF_6	Water fluoridation, disinfectant, wood preservative, masonry hardener, treatment of skins and hides, electroplating, glass treatment
Sodium fluorosilicate	Na_2SiF_6	Opacification of vitreous enamels and opalescent glass, coagulation of latex, treatment of hides and skins, zirconia pigment production, beryllium extraction, insecticide
Potassium fluorosilicate	K_2SiF_6	Chemical intermediate, vitreous enamel frits
Zinc fluorosilicate	$ZnSiF_6 \cdot 6H_2O$	Laundry sour, wood preservative, plaster additive, concrete additive
Silver fluorosilicate	$Ag_2SiF_6 \cdot 4H_2O$	Antiseptic preparations
Barium fluorosilicate	$BaSiF_6$	Opacifier in enamel frits, insecticide, fungicide
Copper fluorosilicate	Cu_2SiF_6	Dyeing and hardening marble, insecticide, fungicide, activating agent in flotation processes
Sodium fluoride	NaF	Rimmed steel manufacture, water fluoridation, heat treating salts, stainless-steel pickling, soldering and metallurgical fluxes, wood preservatives, adhesives, insecticide, coated papers, fluxing agent for vitreous enamels, antiseptic

TABLE 7.1 (continued)

Compound	Formula	Use
Sodium bifluoride	$NaHF_2$	Laundry sour and stain remover, bleach for leathers, tin plating, stone and brick cleaner, glass etching and frosting
Sulfur hexafluoride	SF_6	Electrical equipment
Fluorosulfuric acid	HSO_3F	Catalyst for alkylation of branched chain paraffins and aromatic compounds, polymerization of monoolefins, electropolishing metals
Stannous fluoride	SnF_2	Dental preparations and dentifrices
Uranium tetrafluoride	UF_4	Starting material for production of uranium metal and UF_6
Fluorinated hydrocarbons		Refrigerants, aerosol propellants, fire-extinguishing agents, heat transfer agents
Fluoroethanols		Anesthetics, drugs
Fluoro ethers and amines		Hydraulic fluids, heat transfer media, dielectric fluids, selective solvents
Perchlorofluoroacetones		Oil and water repellent textile coatings, acetal resin solvents, adhesives, heat transfer agents, pesticides
Fluorinated carboxylic acids		Rodenticides, insecticides
Fluorinated aromatic compounds		Lamprey irradicants, dyes, pharmaceuticals, organic solvents, fungistats, anticonvulsants
Perfluoroalkylsulfur fluorides		Dielectric materials, solvents, thermally stable fluids, inert polymers, polymerization initiator
Polytetrafluoroethylene		Chemical gasketing and packing material, valves to control gas and liquid flow, hoses, electronic components, sealants, cooking utensil coatings, bearings
Polychlorotrifluoroethylene		Chemical process equipment, electrical wire and components, organic seals and gaskets, outdoor protective coverings, electrical insulation, printed circuits, sealants, gyroscopic fluids, lubricants
Polyvinyl fluoride		Metal coatings, wood and plastic laminants, fabric coatings, wall coatings, greenhouses and solar stills, mold release films for resins, electrical components
Polyvinylidene fluoride		Piping, coatings for tanks, valves, and pumps, instrument diaphragms, hoses, shipping containers, electronic equipment

Source: Compiled from Standen, 1966.

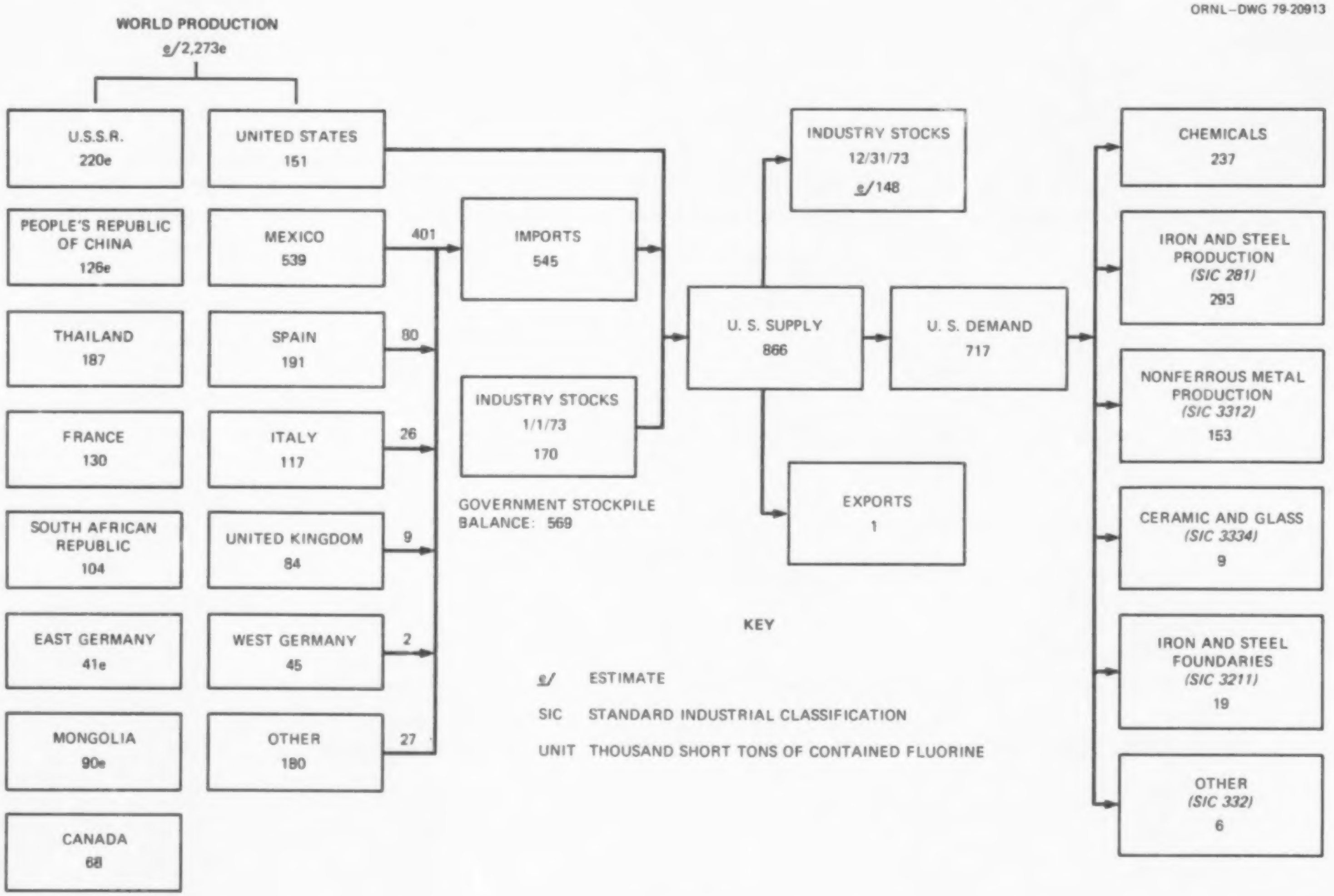
ORNL–DWG 79-20913
WORLD PRODUCTION
e/2,273e
U.S.S.R.
220e
UNITED STATES
151
PEOPLE'S REPUBLIC OF CHINA
126e
MEXICO
539
THAILAND
187
SPAIN
191
FRANCE
130
ITALY
117
SOUTH AFRICAN REPUBLIC
104
UNITED KINGDOM
84
EAST GERMANY
41e
WEST GERMANY
45
MONGOLIA
90e
OTHER
180
CANADA
68
401
80
26
9
2
27
IMPORTS
545
INDUSTRY STOCKS
1/1/73
170
GOVERNMENT STOCKPILE
BALANCE: 569
U. S. SUPPLY
866
INDUSTRY STOCKS
12/31/73
e/148
EXPORTS
1
U. S. DEMAND
717
CHEMICALS
237
IRON AND STEEL PRODUCTION
(SIC 281)
293
NONFERROUS METAL PRODUCTION
(SIC 3312)
153
CERAMIC AND GLASS
(SIC 3334)
9
IRON AND STEEL FOUNDARIES
(SIC 3211)
19
OTHER
(SIC 332)
6
KEY
e/ ESTIMATE
SIC STANDARD INDUSTRIAL CLASSIFICATION
UNIT THOUSAND SHORT TONS OF CONTAINED FLUORINE

Figure 7.1. Supply-demand relationships for fluoride (all forms). Source: Adapted from Wood, 1975. Reprinted by permission of the publisher.

TABLE 7.2. SUMMARY OF FORECASTS OF U.S. AND WORLD FLUORINE DEMAND, 1973-2000 (thousand metric tons of contained fluorine)

Location	1973	2000 forecast range		Probable		Probable average annual growth rate, 1973-2000 (%)
		Low	High	1985	2000	
United States						
Total	717	1,570	2,850	1,570	1,940	3.8
Cumulative		37,000	46,000	13,600	39,800	
Rest of world						
Total	1,556	3,000	7,500	3,640	5,400	4.7
Cumulative		80,000	112,000	30,000	98,000	
World						
Total	2,273	4,570	10,350	5,210	7,340	4.4
Cumulative		117,000	158,000	43,600	137,800	

Source: Adapted from Wood, 1975, Table 10, p. 396. Reprinted by permission of the publisher.

TABLE 7.3. SOLUBLE FLUORIDE EMISSIONS^{a}

Current				Year 2000 with current practice		Year 2000 assuming 99% control efficiency	
Process	Emission (thousands of tons fluoride per year)	Year	Relative confidence^{b}	Process	Emission (thousands of tons fluoride per year)	Process	Emission (thousands of tons fluoride per year)
Coal burning for power	27	1970	II	Primary aluminum manufacture	141	Primary aluminum manufacture	8.1
Open hearth steelmaking	25	1968	II	Coal burning for power	86	Coal burning for power	0.9
Iron ore sintering	18	1968	II	Iron ore pelletizing^{c}	39	HF production	0.7
Iron ore pelletizing^{c}	18	1968	II	Expanded clay aggregate	25	Electrothermal phosphorus	0.4
Primary aluminum production	16	1970	I	Wet-process phosphoric acid^{d}	22	Iron ore pelletizing	0.4
Heavy clay products	10	1968	II	HF alkylation^{e}	16	Wet-process phosphoric acid^{d}	0.3
Wet-process phosphoric acid^{d}	6.4	1970	I	Heavy clay products	10	Triple superphosphate^{d}	0.3
HF alkylation^{e}	5.8	1971	I	Triple superphosphate^{d}	7.3	Expanded clay aggregate	0.3
Expanded clay aggregate	5.3	1968	II	Electrothermal phosphorus	6.6	Defluorinated phosphate rock^{d}	0.2
Normal superphosphate	5.0	1970	I	Opal glass production	5.5	HF alkylation	0.2
Electrothermal phosphorus	4.1	1968	I	HF production	5.3	Normal superphosphate	0.1
Triple superphosphate^{d}	3.8	1970	I	Iron ore sintering	4.8	Heavy clay products	0.1
Opal glass production	3.3	1968	II	Defluorinated phosphate rock^{d}	2.7	Opal glass production	<0.1
Blast furnace	2.8	1968	II	Blast furnace^{f}	2.6	Iron ore sintering	<0.1
Defluorinated phosphate rock^{d}	1.8	1970	I	Normal superphosphate	1.4	Blast furnace	<0.1
HF production	0.7	1970	I	Enamel frit production	1.1	Ammonium phosphate^{d}	<0.1
Enamel frit production	0.7	1968	II	Cement manufacture	0.8	Enamel frit production	<0.1
Copper smelting and refining	0.6	1967	III	Copper smelting and refining	1.6	Copper smelting and refining	<0.1
Ammonium phosphate^{d}	0.3	1970	I	Ammonium phosphate^{d}	0.8	Cement manufacture	<0.1
Cement manufacture	0.3	1964	II	Open hearth steelmaking	0	Open hearth steelmaking	0
Lead smelting and refining	0.2	1967	III	Lead smelting and refining	2.4	Lead smelting and refining	<0.1

TABLE 7.3 (continued)

Current				Year 2000 with current practice		Year 2000 assuming 99% control efficiency	
Process	Emission (thousands of tons fluoride per year)	Year	Relative confidence[b]	Process	Emission (thousands of tons fluoride per year)	Process	Emission (thousands of tons fluoride per year)
Zinc smelting and refining	0.2	1967	III	Zinc smelting and refining	2.4	Zinc smelting and refining	<0.1
Total for processes considered	155.3				384.3		12.0

[a] Excludes CaF_2.
[b] Relative confidence levels: I – excellent, II – good, III – fair to poor.
[c] Assumes no fluorspar addition to pellet.
[d] Includes pro rata allocation of gypsum pond fluoride emissions (estimated as 6300 tons for 1970 and 21,000 tons for 2000).
[e] Assumes 25% of production uses lime pit disposal of acid sludges.
[f] Assumes no limestone other than that in pellets or sinter.

Source: Adapted from Robinson et al., 1972, Table 12. Reprinted by permission of the publisher.

phosphate rock; producing iron, steel, and aluminum; burning coal; and manufacturing glass, ceramic, and clay products (Duncan, Keitz, and Krajeski, 1973; Robinson et al., 1972). Fluorides emitted by these processes are primarily in the form of hydrofluoric acid, silicon tetrafluoride, or particulate matter (National Academy of Sciences, 1974). Major sources of fluoride contamination are discussed extensively in National Academy of Sciences (1971); that account is briefly summarized below.

7.3.1.1.1 Phosphate fertilizer production — Phosphate rock mined in the United States is usually converted to phosphate fertilizers, phosphoric acid, or elemental phosphorus. During the grinding and drying of phosphate rock, dusts and gases containing fluorides may be liberated (Table 7.4). Gypsum settling ponds are also a source of atmospheric pollution (Smith and Hodge, 1978). The chief gaseous pollutant produced is silicon tetrafluoride, although hydrogen fluoride is also emitted (Russell, 1968). In modern plants that practice good emission control, most of these fluorides are removed from off-gases by water-spray towers or other types of scrubbers.

7.3.1.1.2 Wet-process phosphoric acid production — Sodium fluorosilicate, potassium fluorosilicate, hydrofluoric acid, and fluorosilicic acid slurries are produced during the manufacture of phosphoric acid. In some coastal areas these wastes are dumped directly into the sea. In other areas they are accumulated in settling ponds. Volatile fluorides are usually not controlled in these ponds and constitute an atmospheric emission source (Cross and Ross, 1969).

7.3.1.1.3 Brick, tile, pottery, and cement production — Clays used in manufacturing these products usually contain 0.02% to 0.3% fluoride. During the firing of brick, tile, and pottery, hydrogen fluoride and silicon tetrafluoride are produced. During brickmaking, 30% of the initial fluoride in clays may be emitted (Semrau, 1957). Gas and dust from kilns are usually discharged untreated into the atmosphere. Fluoride-containing frit is often used to color ceramics, thus providing another potential emission (Wildblood, 1973).

7.3.1.1.4 Glass, enamel, and fiberglass production — Fluorspar, cryolite, and sodium fluorosilicate are often added to special-purpose glasses. Gaseous and particulate fluorides escape to the environment during this process. Vapor from etching solutions containing hydrofluoric acid or ammonium bifluoride can escape to the atmosphere. Silicon tetrafluoride, hydrogen fluoride, calcium fluoride, aluminum fluoride, sodium fluoride, and sodium fluorosilicate are also produced during glass melting operations; overall, 10% to 20% of the fluoride in the raw materials is emitted to the environment during glass manufacture (Semrau, 1957).

7.3.1.1.5 Metal casting, welding, and brazing — Fluorides are used as fluxes and coatings for molds in metal casting operations. Metal casting produces hydrogen fluoride, silicon tetrafluoride, and boron trifluoride. Efforts are seldom made to reduce atmospheric emissions of this nature.

TABLE 7.4. FLUORINE EMISSIONS FROM PROCESSING PHOSPHATES

Process	Process material	Process equipment	Input fluorine emitted (%)	Remarks
Calcining phosphate rock, beneficiation	Phosphate rock	Rotary kiln	3	Residence time short at maximum temperature
			Almost nil	
Calcining phosphate rock, defluorination	Phosphate rock	Rotary kiln	50-70	Residence time — 1 hr
			75	Residence time — 2 hr
Calcining phosphate rock, defluorination	Phosphate rock, silica	Rotary kiln	98	Residence time in burning zone — 20-30 min
Nodulizing phosphate rock	Phosphate rock	Rotary kiln	20-45	Residence time — 30 min
Sintering phosphate rock	Phosphate rock	Dwight-Lloyd machine	30	Moisture in charge
			35-40	
Calcining phosphate rock	Phosphate rock, briquets	Rotary kiln	Almost nil	
Calcining phosphate rock	Phosphate rock, pellets	Shaft kiln	14	
			14	
		Rotary kiln	28	
Defluorinating molten phosphate rock	Phosphate rock, silica	Electric furnace	Small	Melting without further treatment
		Hearth furnace	84-95	Melting time — 4-25 hr
		Hearth furnace	72-86	Pool depth — 18 in.
		Electric furnace (for melting)	90	Pool depth — 3 in., heating period — 2 hr
		Hearth furnace (for defluorination)	92-96	Pool depth — 1.5-2.0 in., heating period — 60-80 min
		Shaft furnace	70	
			90 or greater	
Phosphoric acid manufacture	Phosphate rock, coke	Electric furnace	35	
			25	
			20-30	
	Phosphate rock, silica	Blast furnace	52	About 17% of emission in particulate form
			30	About 50% of emission in particulate form
Elemental phosphorus manufacture	Phosphate rock, silica and coke	Electric furnace	9.6	About 16% of emission in particulate form
Calcium-magnesium phosphate manufacture	Phosphate rock, olivine	Electric furnace	27-33	Batch operation
			11	Continuous charging
	Phosphate rock, olivine and silica	Electric furnace	27-57	Batch operation
	Phosphate rock, olivine	Electric furnace	10-20	Fusion time — 1-2 hr Continuous charging with unfused material above melting zone
Manufacture of Rhenania phosphate	Phosphate rock, soda ash and silica	Rotary kiln	8	
Manufacture of calcium metaphosphate	Phosphate rock	Combustion chamber and absorption tower	80-85	
Defluorination of superphosphate	Triple superphosphate	Rotary kiln	78-82	Residence time — 27 min
Manufacture of superphosphate	Phosphate rock, sulfuric acid	Mixer and den	16-42	Extreme range
			30-35	Average

Source: Adapted from Semrau, 1957, Table V, p. 98. Reprinted by permission of the publisher.

Calcium fluoride or other inorganic fluorides are often used as fluxing agents for arc welding. Hydrogen fluoride, silicon tetrafluoride, and particulate fluorides are released during the welding process (Pantucek, 1971). Fluoride concentrations of up to 10 mg per cubic meter of air may be produced by welding in confined areas (Smith, 1967). Such concentrations exceed the fluoride threshold limit value of 2.5 mg/m^3 for particulate fluorides set by the American Conference of Governmental Industrial Hygienists. Silver soldering and brazing also release fluoride particulates.

7.3.1.1.6 Phosphate and phosphate feed supplement production — Silicon tetrafluoride, hydrogen fluoride, and fluoride dusts are formed during phosphate manufacture. Modern plants usually remove these pollutants by spray towers or scrubbers. Slag from production furnaces contains about 3% fluoride and is usually solidified or sold as an agricultural liming agent. Hydrogen fluoride is also produced during the manufacture of animal feed supplements; however, emissions from this process are small.

7.3.1.1.7 Aluminum production — Aluminum electrolysis cells emit silicon tetrafluoride, hydrogen fluoride, cryolite, aluminum fluoride, calcium fluoride, and chiolite. Although aluminum manufacture has constituted a significant source of emissions in the past, ambient air fluoride concentrations from aluminum plants are now generally low (Schmitt, 1963; Smith and Hodge, 1978).

7.3.1.1.8 Steel production — Fluorides constitute only a fraction of the fumes produced by steel manufacturing. Primarily, calcium fluoride and hydrogen fluoride are produced. These fluorides are usually efficiently removed from off-gases, resulting in relatively low concentrations of fluoride in gaseous emissions. As in aluminum production, however, the aggregate total weight released is substantial (Smith and Hodge, 1978).

7.3.1.1.9 Petroleum refining — Boron trifluoride and hydrogen fluoride are used by the petroleum industry as alkylation catalysts in the production of high-octane gasolines. Volatile fluorides may be released during loading and unloading of chemicals and during disposal of waste products. Tarry residues that release fluoride fumes when exposed to air are frequently produced.

7.3.1.1.10 Rocket engine testing — Fluorine and its compounds are used as oxidizers in rocket fuels, and testing of rocket engines results in fluoride emissions. Contamination may also result from burned purge gases and discharges from pipe burnouts and spills (Ricca, 1966).

7.3.1.1.11 Pesticide application — Among fluoride compounds used as pesticides are sodium fluoride, sodium fluorosilicate, barium fluorosilicate, cryolite, and various organic fluorides. Use of fluoride-containing products has declined in recent years due to the development of organic phosphates. Some uses, however, may still provide a direct environmental emission.

7.3.1.1.12 Coal combustion — Coal combustion provides a significant environmental input of fluoride. U.S. coals contain 25 to 143 ppm fluoride, with an average of 60.9 ppm (Ruch, Gluskoter, and Shimp, 1974). Abernethy and Gibson (1963) reported 10 to 190 ppm fluoride in U.S. commercial coals. British coals contain 0 to 175 ppm fluoride, most containing less than 80 ppm (Crossley, 1944). A global average of 80 ppm in coal is suggested by Bowen (1966). During combustion, about 50% of the fluoride contained in coal is evolved as hydrogen fluoride, silicon tetrafluoride, and particulates.

7.3.1.1.13 Atomic energy installations — Gaseous diffusion plants use uranium hexafluoride to separate isotopes of uranium. Due to strict control measures, emission of fluoride compounds is usually negligible, except in cases of accidental release (Emler, Hulett, and Kalmon, 1971).

7.3.1.1.14 Other — Additional industrial emission of fluorides results from quartz etching, metal pickling, electroplating, addition of fluorides to water, toothpastes, bleaches, drugs, incineration of wastes, and mining (Waldbott and Oelschlager, 1974; Wood, 1973).

7.3.1.2 Natural Sources — Natural sources of fluorides include minerals and rocks in the earth's crust, precipitation, volcanoes, and fumaroles. The fluoride content of minerals and rocks is discussed in Section 7.3.2.1.

7.3.1.2.1 Volcanoes and fumaroles — Gaseous and particulate fluorides are discharged by active volcanoes and fumaroles. Fluoride compounds in volcanic gases include hydrogen fluoride, ammonium fluoride, silicon tetrafluoride, ammonium fluorosilicate, sodium fluorosilicate, potassium fluorosilicate, and potassium fluoroborate. Fluorides of potassium, sodium, calcium, and magnesium have also been identified, as well as trace amounts of organofluorine compounds (Roholm, 1937; Stoiber et al., 1971). Volcanism adds an estimated 1 to 7 × 10^9 kg of fluoride per year to the environment (Bartels, 1972; Carpenter, 1969).

7.3.1.2.2 Precipitation — Precipitation collects atmospheric fluoride and returns it to earth, providing a localized input (Table 7.5). Rainwater in industrialized areas contains more fluoride than that in nonindustrialized areas. MacIntire et al. (1949) found that rainwater averaged 0.29 ppm in a city, 0.30 ppm near a phosphate processing plant, and 0 to 0.02 ppm in a control area. Rainwater in a German industrial area averaged 0.28 to 14.1 ppm (Garber, 1970). In areas where much coal is burned, rainwater adds an estimated 170 g of fluoride per hectare of land (MacIntire et al., 1942). Global precipitation is estimated to provide 1.2 × 10^{10} kg (2.6 × 10^{10} lb) of fluoride per year (Bewers and Haysom, as cited in Bewers and Yeats, 1975).

7.3.2 Distribution in Rocks and Soils

7.3.2.1 Minerals and Rocks — Fluoride in the lithosphere is primarily associated with minerals, usually phosphates and silicates. The most abundant fluoride mineral is fluorite (fluorspar, CaF_2). Other economically important fluoride minerals are cryolite (Na_3AlF_6), sellaite (MgF_2),

TABLE 7.5. FLUORIDE CONCENTRATIONS IN ATMOSPHERIC PRECIPITATION

Location	Type	Number	Fluoride (ppm)	
			Range	Mean
Japan	Rain	1		0.06
England	Rain	9	0.08-0.54	0.22
California	Rain	35	0.0-0.8	0.12
		12	0.02-0.57	0.14
Mojave Desert	Rain	4	0.0-0.7	0.2
Siberia	Snow	5	0.05-0.30	0.13
Kazakistan	Snow	4	0.05-0.20	0.10
Ukraine	Rain			0.052
Sierra Nevada	Snow	28	0.0-0.2	0.06
Czechoslovakia	Snow	40		0.36
Minnesota	Snow			
	Fresh urban		0.03-0.04[a]	
				0.05[b]
	Wilderness		[c]	0.05[b]
	Street, after several weeks		1.62-3.27[b]	0.45[a]
	Rain			
	Fresh urban			0.7[a]
				0.22[b]
	Snow runoff after above rain			0.37[a] 2.41[b]
Storm at sea, east of Newfoundland	Rain			0.05
Unspecified	Rain		0.001-1	
Calcutta	Premonsoon showers		0.02-0.4	
	Rain			
	Collected over 12 months near fertilizer factory			
	500 m ENE		5.3-14.1	10.0
	500 m SW		0.79-3.3	1.85
	1000 m NE		3.1-6.3	5.1
	Collected over 3 months close to another fertilizer factory, three sites		0.61-1.6	
	Control		0.16	
	Collected over 12 months near industrial area of Hamburg			
	Near		0.41	
	1000 m NE		0.41	
	1800 m		0.28	
	3000 m N		0.61	
	Control		0.19	

[a] Ionic fluoride.
[b] Total fluoride.
[c] Ionic fluoride not detected.

Source: Adapted from Smith and Hodge, 1978, Table 7, p. 298. Reprinted by permission of the publisher.

villiaumite (NaF), bastnaesite [$(Ce,La)(CO_3)F$], fluorapatite [$Ca_5(PO_4,CO_3)_3F$], and topaz [$Al_2SiO_4(F,OH)_2$] (Worl, Van Alstine, and Shawe, 1973).

Virtually all rocks contain fluoride. Igneous rocks average 210 to 1000 ppm fluoride, and sedimentary rocks average 180 to 940 ppm (Fleischer and Robinson, 1963). Hawkes and Webb (as cited in Shacklette, Boerngen, and Keith, 1974) supplied the following values: ultramafic rock, 100 ppm; mafic, 370 ppm; felsic, 800 ppm; limestone, 51 ppm; sandstone, 290 ppm; and shale, 590 ppm. Kokuba (as cited in Shacklette, Boerngen, and Keith,

1974) reported 100 to 800 ppm fluoride in volcanic rocks and 120 to 2400 ppm in plutonic rocks. Glass-rich volcanic rocks of the western United States contain 20 to 4900 ppm fluoride (Coats, Goss, and Rader, 1963). Bowen (1966) gave the fluoride content of igneous rocks as 625 ppm, sandstone as 270 ppm, shales as 740 ppm, and limestone as 330 ppm.

7.3.2.2 Soil — Estimates of fluoride in the earth's crust range from 300 ppm (Largent, 1961) to 800 ppm (Murrmann and Koutz, 1972). Normal mineral soils average 200 to 300 ppm fluoride (Bowen, 1966; Worl et al., 1973). Of this, 10 to 40 ppm is extractable, and the concentration in the soil solution is 0.1 to 0.5 ppm (Murrmann and Koutz, 1972). Generally, sand soils are low in fluoride, while heavier soils contain higher concentrations. The high incidence of micaceous minerals and their weathering products in clays probably accounts for the high fluoride content of the latter (Table 7.6).

TABLE 7.6. FLUORIDE CONTENT OF CERTAIN MICACEOUS CLAYS

Mineral	Location	Fluoride (ppm)
Hydrous mica	Platteville, Wis.	5800
Muscovite	Staley, N.C.	400
Ordovician bentonite	Chattooga County, Ga.	4500
	Sevier Dam, Tenn.	7400
Sericite	Guanajuato, Mexico	1800
	Staley, N.C.	300

Source: Adapted from Robinson and Edgington, 1946, Table 2, p. 347. Reprinted by permission of the publisher.

Soils of the eastern United States contain 10 to 3680 ppm fluoride (arithmetic mean, 340 ppm) while those of the western United States contain 10 to 1900 ppm (arithmetic mean, 410 ppm) (Figure 7.2). The difference in fluoride content between the eastern and western areas is statistically significant (1% level) and is probably due to differences in the fluoride content of the parent materials (Shacklette, Boerngen, and Keith, 1974). Robinson and Edgington (1946) analyzed 30 profiles of representative U.S. soils (Table 7.7). The fluoride content ranged from a trace (undefined) to 7070 ppm. The average fluoride content to plow depth (about 15.2 cm, or 6 in.) was 292 ppm. Calcium chloride-extractable fluoride in United Kingdom soils usually ranges from 0 to 0.2 ppm (Larsen and Widdowson, 1971). Labile (resin-extractable) fluoride averaged about 20 ppm. The fluoride content varied with depth in some soils.

Soils rich in phosphorus tend to contain higher-than-normal fluoride levels. A high-phosphate Tennessee soil contains as much as 0.83% fluoride

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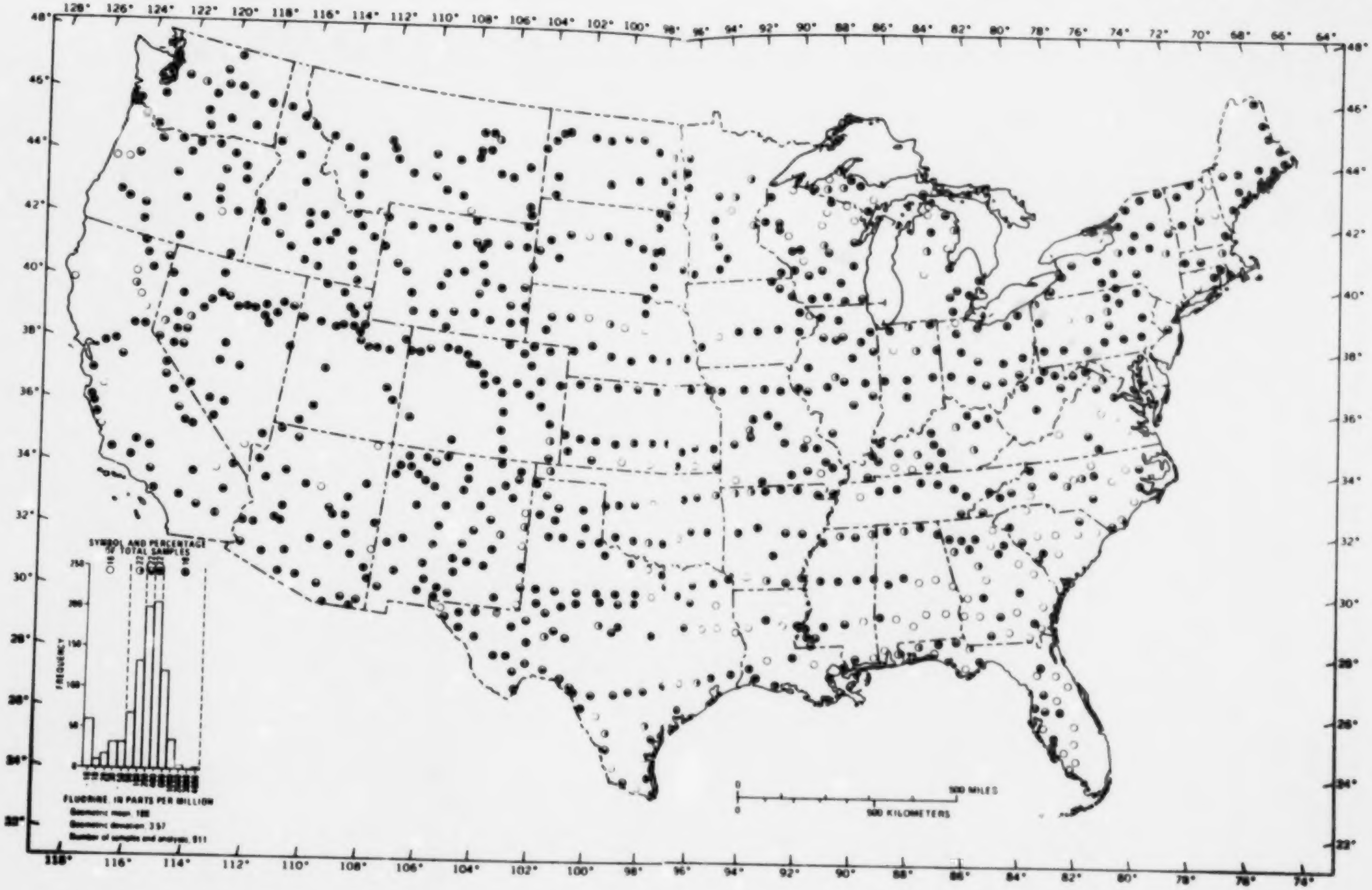
SYMBOL AND PERCENTAGE OF TOTAL SAMPLES
FREQUENCY
250
200
150
100
50
0
FLUORINE, IN PARTS PER MILLION
Geometric deviation: 3.57
Number of samples and analyses: 911
500 MILES
500 KILOMETERS
0
48°
46°
44°
42°
40°
38°
36°
34°
32°
30°
28°
26°
24°
22°
126°
124°
122°
120°
118°
116°
114°
112°
110°
108°
106°
104°
102°
100°
98°
96°
94°
92°
90°
88°
86°
84°
82°
80°
78°
76°
74°
72°
70°
68°
66°
64°

Figure 7.2. Fluorine concentrations in surficial materials of the conterminous United States. Source: Shacklette, Boerngen, and Keith, 1974, Figure 3, pp. 8-9.

TABLE 7.7. FLUORIDE IN SOILS

Soil type	Location	Depth (in.)	Fluoride (ppm)
Arredonda fine sand	Gainesville, Fla.	0-36	20
Barnes loam	La Bolt, S.D.	0-9	220
		9-17	232
		17-33	357
		33-60	390
Brassua sandy loam	North Grafton, N.H.	0-3	22
		3-4	70
		4-9	62
		9-19	97
		19+	92
Bridgeport loam	Sheridan, Wyo.	0-3	270
		3-7	335
		7-15	388
		15-20	440
		20-27	487
		27-34	461
Carrington loam	Winthrop, Iowa	0-3	120
		3-13	127
		13-22	130
		22-43	190
		43-70	150
		70-84	210
Chester silt loam	Rockville, Md.	0-1	170
		1-9	175
		9-20	250
		20-26	307
		38-41	380
	Falls Church, Va.	0-8	137
		12-21	172
Unidentified clay loam	Caribou County, Idaho	0-12	1610
		12-24	3870
Colby silty clay loam	Hays, Kan.	0-10	ND[a]
		10-20	403
		20-33	495
		33-47	522
		47-60	525
		60-72	491
Fayette silt loam	LaCross, Wis.	0-8	187
		8-20	215
		20-32	246
		33-44	280
		44-66	232

TABLE 7.7 (continued)

Soil type	Location	Depth (in.)	Fluoride (ppm)
Frederick silt loam	Fairfield, Va.	0-2	207
		2-11	145
		11-16	287
		16-36	1032
		36-56	1305
Greenville sandy loam	Pretoria, Ga.	½-2	28
		2-7	52
		7-15	38
		15-24	44
		24-42	93
		42-72	113
		72-108	184
Hagerstown silt loam	State College, Pa.	0-2	260
		2-8	310
		14-35	720
		35-46	902
	Hagerstown, Md.	0-3	332
		3-12	352
		12-33	1832
		33-48	1910
Holdredge silt loam	Custer County, Neb.	0-2	240
		2-12	285
		12-24	432
		24-36	490
		36-60	432
		60-72	457
Houston black clay	Temple, Tex.	0-3	372
		11-20	416
		24-36	425
		36-50	364
Kalkaska loamy sand	Alger County, Mich.	0-2	20
		2-6	Trace
		6-24	12
		24-48	15
Kirvin fine sandy loam	Tyler, Tex.	0-12	48
		12-24	334
		24-51	299
		51-63	133
		63-75	58
Madison sandy loam	Gainesville, Ga.	0-6	234
		6-18	269
		18-30	235
		30-36	300
		36-40	334

TABLE 7.7 (continued)

Soil type	Location	Depth (in.)	Fluoride (ppm)
Marshall silt loam	Clarinda, Iowa	0-13	376
		13-24	411
		24-45	394
		45-71	296
Maury silt loam	Ashwood, Tenn.	0-2	506
		2-12	290
		12-25	850
		25-40	7070
		40-60	4300
		60-90	4080
Miami silt loam	Wayne County, Ind.	0-2	95
		2-5	130
		5-11	135
		11-15	187
		15-30	400
		30-36	452
Muskingum silt loam	Zanesville, Ohio	0-7	316
		8-13	390
		14-24	344
		25-46	326
		47-72	365
Norfolk sandy loam	Bests, Wayne County, N.C.	0-12	42
		12-34	70
		36-80	95
Oahu clay	Oahu, Hawaii	0-10	120
		10-25	87
		25-40	55
Palouse silt loam	Pullman, Wash.	0-20	369
		20-33	364
		33-62	360
		62-75	389
		75-84	358
Shelby silt loam	Bethany, Mo.	0-7	276
		8-12	444
		12-20	487
		20-24	468
		24-48	438
		48-60	442
		48-	306
		60-84	420

TABLE 7.7 (continued)

Soil type	Location	Depth (in.)	Fluoride (ppm)
Redding clay loam	San Diego County, Calif.	0-7	85
		7-14	106
		14-24	130
		24-33	105
		33-41	146
		41-51	154
Sharkey clay	Houma, La.	0-6	590
		10-24	525
		48-80	620
Vernon fine sandy loam	Guthrie, Okla.	0-3	40
		3-10	85
		10-27	183
		27-58	293

[a] Not determined.

Source: Adapted from Robinson and Edgington, 1946, Table 1, p. 343. Reprinted by permission of the publisher.

(MacIntire et al., 1949). Soils receiving annual applications of fertilizers, particularly superphosphates, have increased fluoride content. Superphosphates contain about 1% to 3% fluoride (Evans, Hoyle, and MacAskill, 1971; Larsen and Widdowson, 1971), 90% of which may accumulate in soil (Robinson and Edgington, 1946). Annual application of fertilizers to German soils has added 8 to 20 kg of fluoride per hectare (7.1 to 17.8 lb/acre) (Oelschläger, 1971). Table 7.8 shows typical fluoride concentrations of German fertilizers. The addition of 453.6 kg (1000 lb) of superphosphate to 0.4 ha (1 acre) of soil can increase the fluoride content of the plow layer by 7.5 ppm (Robinson and Edgington, 1946). Application of 500 kg (1102.3 lb) of superphosphate per hectare (2.47 acres) raised soil fluoride content by about 7 ppm in New Zealand (Stewart et al., 1974). Hamilton (1974) estimated that annual fertilizer applications provide an additional 5 to 30 kg of fluoride per hectare per year (4.5 to 26.8 lb/acre per year), of which 30 to 400 g (0.6% to 1.3%) is leached annually.

Soils near industrialized areas show elevated fluoride content due to contamination. The fluoride concentration in the top 1.3 cm (0.5 in.) of soil at a phosphorus extraction facility near Silverbow, Montana, ranges from 265 to 1840 ppm (van Hook, 1974). Fluoride content decreases with increasing distance from the facility. Oelschläger (1971) found that 2.1 kg of fluoride per hectare per year (1.9 lb/acre per year) was accumulated in soils in a large industrialized area.

TABLE 7.8. FLUORIDE CONTENT OF PRINCIPAL GERMAN FERTILIZERS

Fertilizer	Fluoride (g/kg)
Rohphosphate	33.4-41.1
Hyperphos	31.0-36.4
Rhenaniaphosphat	15.0-27.7
Superphosphat	14.6-25.7
Thomasphosphat	0.01-0.14
Branntkalk	0.05-0.19
Mergel	0.06-0.27
Dolomitkalk	0.22
Hüttenkalk	3.9-10.2
Kalidüngesalz (40%)	0.02-0.03
Kainit	0.01-0.02
Schwefelsaures ammoniak	0.003
Kalkammonsalpeter	0.01
Kalkstickstoff	0.58
Stallmist (fresh manure)	0.003

Source: Adapted from Oelschlager, 1971, Table 1, p. 81. Reprinted by permission of the publisher.

7.3.3 Distribution in Water

Fluoride is a normal constituent of natural waters. Uncontaminated surface waters (rivers, lakes, ponds, canals, creeks, and cisterns) usually do not exceed a fluoride content of 0.3 mg/liter (Maier, 1972). The fluoride content of surface waters varies depending on the source of the water and the amount of precipitation received (Worl, Van Alstine, and Shawe, 1973). Table 7.9 gives the fluoride content of North American waters. Livingston (1963) gave the fluoride content of world rivers as 0.1 to 0.2 ppm. Bowen (1966) suggested an average of 0.09 ppm. The fluoride content of drinking water from many different countries is given in Table 7.10. Although some of the extreme values are very high, average fluoride concentrations near 1 ppm are common.

Groundwaters (springs, wells, and infiltration galleries) may contain as much as 67.2 ppm fluoride, but few contain more than 10 ppm (Worl et al., 1973). Large areas of the world have local groundwaters with more than 1.5 ppm fluoride. The fluoride content of groundwaters depends, in part, on the nature of the rock through which they flow; alkalic igneous rocks, dolomite, phosphorite, and volcanic glasses give rise to high-fluoride waters (Livingstone, 1963; Worl, Van Alstine, and Shawe, 1973). Groundwaters are the source of most U.S. water supplies.

Fluorides are, of course, added to many community water supplies to help decrease the incidence of dental caries. Maier (1972) estimated that

TABLE 7.9. FLUORIDE IN NORTH AMERICAN WATERS

Location	Date	Average fluoride (ppm)
Ouachita River near Malvern, Ark.	Oct. 1946-Sept. 1947	0.3
Ouachita River at Arkadelphia, Ark.	Oct. 1959-Sept. 1960	0.2
Smackover Creek near Smackover, Ark.	Oct. 1949-Sept. 1950	0.1
Salton Sea, Imperial Valley, Calif.	Feb. 1954	1.6
Mono Lake, Mono County, Calif.	Sept. 1956	23
Rush Creek near Mono Lake, Calif.	Oct. 1953	0.2
Pons at Badwater, Death Valley, Calif.	Apr. 1964	4.9
Amargosa River near Beatty, Nev.		4.8
Lake Tahoe at Bijou, Nev.	Sept. 1953	0.1
Truckee River at Farad, Calif.	Sept. 1953	0.1
Pyramid Lake at Sutcliffe, Nev.	Sept. 1955	0.8
Eagle Lake near Susanville, Calif.	May 1954	7.0
Lower Alkali Lake near Eagleville, Calif.	May 1954	10.0
Middle Alkali Lake near Cedarville, Calif.	May 1954	6.0
Devils Lake, N.D.	Nov. 1948 July 1950	1.4 0.6
Black Tiger Bay, Township 152, N.D.		1.4
Dry Lake, Township 155, N.D.		0.2
Free Peoples Lake, Township 151, N.D.		0.8
Round Lake, Township 153, N.D.		0.6
Stink Lake, Township 155, N.D.		3.4
Kootenai River at Porthill, Idaho	Oct. 1949-Sept. 1950	0.2
Flathead River at Columbia Falls, Mont.	Oct. 1949-Sept. 1950	0.2
Pend Oreille River at Metaline Falls, Mont.	Oct. 1949-Sept. 1950	0.3
Columbia River above Dallas, Wash.	Dec. 1958	0.5
Ikrowik Lake near Point Barrow, Alaska	June 1951 July 1951	0.1 0.1
East Oumalile Lake near edge of Arctic Coastal Plain	July 1951	0.1
Chandler Lake, Brooks Range, Alaska	Aug. 1951	0.4
Yukon River at Eagle, Alaska	Apr. 1951-Sept. 1951	0.02
Ohio River at Dam 31		0.3
Ohio River at Dam 39		0.5
Ohio River at Dam 43		0.3
Ohio River at Shawneetown, Ill.		0.4
Ohio River at Dam 53		0.4
Allegheny River at Warren, Pa.	Oct. 1948-Sept. 1949	0.1

TABLE 7.9 (continued)

Location	Date	Average fluoride (ppm)
Clarion River near Piney, Pa.	Oct. 1946-Sept. 1947	0.1
Kiskiminetas River at Leechburg, Pa.	Oct. 1946-Sept. 1947	0.2
Casselman River at Harvedsville, Pa.	Oct. 1949-Sept. 1950	0.1
Youghiogheny River at Sutersville, Pa.	Oct. 1947-Sept. 1948	0.1
Mahoning River at Warren, Ohio	Oct. 1946-Sept. 1947	0.3
Tuscarawas River at Newcomerstown, Ohio	Oct. 1946-Sept. 1947	0.5
Iowa River at Iowa City, Iowa	Oct. 1949-Sept. 1950	0.2
Cedar River at Cedar Rapids, Iowa	Oct. 1949-Sept. 1950	0.1
Wind River at Dubois, Wyo.	Oct. 1948-Sept. 1949	0.3
Wind River at Riverton, Wyo.	Oct. 1948-Sept. 1949	0.2
Bighorn River at Thermopolis, Wyo.	Oct. 1948-Sept. 1949	0.3
Little Missouri River at Medora, N.D.	Oct. 1948-Sept. 1949	0.2
Heart River near South Heart, N.D.	Oct. 1947-Sept. 1948	0.3
Grand River at Shadehill, S.D.	Oct. 1948-Sept. 1949	0.3
Cheyenne River near Hot Springs, S.D.	Oct. 1948-Sept. 1949	0.6
White River near Kadoka, S.D.	Oct. 1949-Sept. 1950	0.6
South Platte River at Juelsburg, Colo.	Oct. 1948-Sept. 1949	0.9
Republican River at Trenton, Kan.	Oct. 1948-Sept. 1949	1.1
Saline River near Wilson, Kan.	Feb. 1948-Sept. 1950	0.4
St. Francis River at Marked Tree, Ark.	Nov. 1949-Sept. 1950	0.1
White River at Batesville, Ark.	Oct. 1945-Sept. 1946	0.1
Black River at Black River, Ark.	Oct. 1945-Sept. 1946	0.1
White River at Newport, Ark.	Oct. 1949-Sept. 1950	0.0
Little Red River near Heber Springs, Ark.	Nov. 1949-Sept. 1950	Trace
Cimmarron River at Ute Park, N.M.	Oct. 1949-Sept. 1950	0.4
Arkansas River at Dardanelle, Ark.	Oct. 1948-Sept. 1949	0.2
Mississippi River near Baton Rouge, La.	Mar. 1959	0.1
Lehigh River at Catasauqua, Pa.	Oct. 1944-Sept. 1945	0.1
Delaware River at Dingmans Ferry, Pa.	Oct. 1944-Sept. 1945	0.2
Delaware River at Belvedere, N.J.	Oct. 1944-Sept. 1945	0.1
Delaware River at Trenton, N.J.	Oct. 1944-Sept. 1945	0.1
West Branch Susquehanna River at Lock Haven, Pa.	Oct. 1945-Sept. 1946	0.1
Frankstown Branch, Juniata River at Huntingdon, Pa.	Oct. 1947-Sept. 1948	0.1
Codorus Creek near York, Pa.	Oct. 1948-Sept. 1949	0.1
Susquehanna River at Conowingo, Md.	Sept. 1958	0.0

TABLE 7.9 (continued)

Location	Date	Average fluoride (ppm)
Hudson River at Green Island, N.Y.	Oct. 1958	0.0
Lake Erie at Huron, Ohio	Sept. 1950-Sept. 1951	0.1
St. Lawrence River at Levis, Quebec	Aug. 1958	0.0
Cuyahoga River at Batzum, Ohio	Oct. 1946-Sept. 1947	0.2
Savannah River near Clyo, Ga.	May 1938-Apr. 1939	0.0
Altamaha River at Doctortown, Ga.	May 1937-Apr. 1938	0.0
Kissimmee River near Okeechobee, Fla.	Mar. 1940-Feb. 1941	0.2
West Palm Beach at Loxahatchee, Fla.	Mar. 1940-Feb. 1941	0.2
Hillsboro Canal at Sharvano, Fla.	Oct. 1950-Sept. 1951	0.5
Withlacoochee River near Holder, Fla.	Oct. 1950-Dec. 1951	0.1
Flint River at Bainbridge, Ga.	Oct. 1941-Sept. 1942	0.1
Chattahoochee River at Columbus, Ga.	Oct. 1940-Sept. 1941	0.1
Apalachicolu River near Blounstown, Fla.	Dec. 1958	0.1
Conasauga River at Tifton, Ga.	Oct. 1942-Sept. 1943	0.0
Oostanaula River at Rome, Ga.	Oct. 1941-Sept. 1942	0.1
Etowah River near Catersville, Ga.	Oct. 1938-Sept. 1939	0.0
Mobile River at Mt. Vernon Landing, Ala.	Dec. 1958	0.1
Ohio River at South Heights, Pa.	Oct. 1945-Sept. 1946	0.2
Ohio River at Dam 13	Sept. 1950	0.3
Ohio River at Dam 19		0.3
Tanana River near Tok Junction, Alaska	Mar. 1951-Sept. 1951	0.02
Tanana River at Big Delta, Alaska	Oct. 1950-Sept. 1951	0.1
Yukon River at Mountain Village, Alaska	Jan. 1959	0.1
Susitna River at Gold Creek, Alaska	May 1951-Sept. 1951	0.2
Eklutna Creek near Palmer, Alaska	Dec. 1950-Sept. 1951	0.1
Ship Creek near Anchorage, Alaska	Oct. 1950-July 1951	0.1
Brown Slough at Bethel, Alaska	Sept. 1951	0.4
Kenai River at Cooper Landing, Alaska	Oct. 1951-Nov. 1951	0.1
Gold Creek, Juneau, Alaska	Oct. 1948-July 1949	0.1
Mackenzie River, Northwest Territory, Canada	July 1958	0.0

Source: Compiled from Livingstone, 1963, Tables 10-12, 25, and 26, pp. G13-G20.

TABLE 7.10. THE OCCURRENCE OF FLUORIDE IN DRINKING WATER FROM VARIOUS COUNTRIES

Location	Fluoride (ppm)	
	Average	Range
North America		
United States		
Alabama		0-6.8
Arizona		<1.0-33.5
Arkansas	0.10	0.0-3.0
California		0-7
Colorado		0-5
Connecticut	0.12	0-0.4
Delaware	0.09	0-0.2
Florida	0.21	0-2.5
Georgia	0.20	0.0-2.0
Hawaii		0-2.22
Idaho		0.3-5.0
Illinois	0.49	0-4.0
Indiana	0.25	0-2.0
Iowa		0-2.8
Kansas		0-10.0
Kentucky	0.5	0-3.9
Louisiana		0-6.0
Maine	0.11	0-0.4
Maryland	0.04	0-5
Massachusetts	0.12	0-8
Michigan	0.22	0-2.0
Minnesota	0.16	0-3.2
Mississippi		0-6.0
Missouri		0-5.0
Montana		[b]
Nebraska		0-2
Nevada		0-5
New Hampshire	0.11	0-0.5
New Jersey	0.08	0-2.1
New Mexico		0-12.0
New York	0.09	0-1.3
North Carolina	0.13	0-4.2
North Dakota		0-7.5
Ohio	0.17	0-3.0
Oklahoma		0-2.4
Oregon	0.03	0-0.5
Pennsylvania	0.06	0-2.0
Rhode Island	0.08	0-0.8
South Carolina	0.48	0-7.0
South Dakota		0-1.2
Tennessee		0-2.0
Texas		0-7.2
Utah		0-5
Vermont	0.02	0-0.1
Virginia	0.12	0-7.5
Washington		0-5.0
West Virginia	0.14	0-0.6
Wisconsin		0-2.8
Wyoming		0.1-1.2
Canada		0-1.2
Mexico		0.1-17.5
Cuba		0-0.4
South America		
Argentina		0-1.6
Brazil		0-0.60
Ecuador		0-6.3
Peru		0-1.4
Europe		
Austria		0.38-0.8
Belgium		0-1.68
Cyprus		0-3.6
Czechoslovakia		0-28.00
England		0-4.5
Finland	0.57	
France		0-7
Germany		0-4.90
Hungary		0-5.14
Ireland		0-0.2
Italy and Sicily		0-7.1
Luxembourg		0.02-1.23
Netherlands		0-2
Norway		0.01-0.07
Poland		0-1.1
Portugal		-22.8[a]
Russia		0-4.6
Spain		0.0-6.3
Sweden		0.0-1.12
Switzerland		0.01-1.42
Yugoslavia		0-4.16
Africa		
South Africa		Trace-53.0
East Africa (Kenya)		0-<30
Tanganyika		Trace-95
Asia		
China		0-13.0
India		0->3.0
Japan		0.02-20.0
Korea		0.8-10.0
Israel		0.3-1.50
Iran		0.0-<1.0
Australia		0.0-13.5
New Guinea		0.0-<0.55
Papua		0.0-<0.55
New Zealand		0-0.9

[a] Mineral waters only.

[b] No records.

Source: Adapted from Cholak, 1959, Table VII, p. 505. Reprinted by permission of the publisher.

83 million persons were being served by artificially fluoridated systems in the United States as of 1970. The U.S. Public Health Service limit for fluoride in drinking waters varies, based on the annual average maximum daily air temperature (Table 7.11), because water consumption increases in warmer weather. Most U.S. water supplies (Figure 7.3) meet the U.S. Public Health Service criteria for fluoride. Eastern and central U.S. water supplies sampled in the 1940s contained a mean of 0.3 ppm (Kehoe, Cholak, and Largent, 1944). A 1962 survey (Durfor and Becker, 1964) of public water supplies of the 100 largest U.S. cities showed 0.0 to 7.0 ppm fluoride, with a mean of 0.4 ppm. Of the systems sampled, 92% contained less than 1 ppm. In 1970, McCabe et al. (1970) found that 52 of 969 U.S. supply systems exceeded the recommended limit, and 24 exceeded the mandatory limit. The maximum fluoride concentration encountered was 4.4 ppm.

TABLE 7.11. U.S. PUBLIC HEALTH SERVICE RECOMMENDED LIMITS FOR FLUORIDE CONCENTRATION

Annual average of maximum daily air temperatures (°F)	Recommended fluoride control limits (mg/liter)		
	Lower	Optimum	Upper
50.0-53.7	0.9	1.2	1.7
53.8-58.3	0.8	1.1	1.5
58.4-63.8	0.8	1.0	1.3
63.9-70.6	0.7	0.9	1.2
70.7-79.2	0.7	0.8	1.0
70.3-80.5	0.6	0.7	0.8

Source: Adapted from U.S. Department of Health, Education, and Welfare, 1962, Table 1, p. 38.

Seawater usually contains more fluoride than freshwater, averaging about 1.4 to 1.5 ppm in waters of normal salinity (Bowen, 1966; Carpenter, 1969; Goldschmidt, 1954; Riley, 1965). Waters that are highly saline exhibit increased fluoride concentrations. The Great Salt Lake, Utah, contains 14 ppm fluoride. Highly saline lakes in Kenya, Africa, contain up to 1627 ppm (Worl, Van Alstine, and Shawe, 1973).

Thermal waters are also enriched in fluoride. Hot waters in areas of recent volcanism, with pH 5 to pH 9, contain 1 to 12 ppm fluoride (Mahon, 1964). Warmwater springs also have an upper limit of about 12 ppm. Acid springs in active volcanic areas may contain 5000 to 6000 ppm fluoride.

Sediments often become a sink for fluoride. Total fluoride in recent oceanic sediments ranged from 450 to 1100 ppm (Carpenter, 1969), with an average of about 730 ppm (Worl, Van Alstine, and Shawe, 1973). The fluoride contents of sediments from the Pacific, Atlantic, and Indian oceans

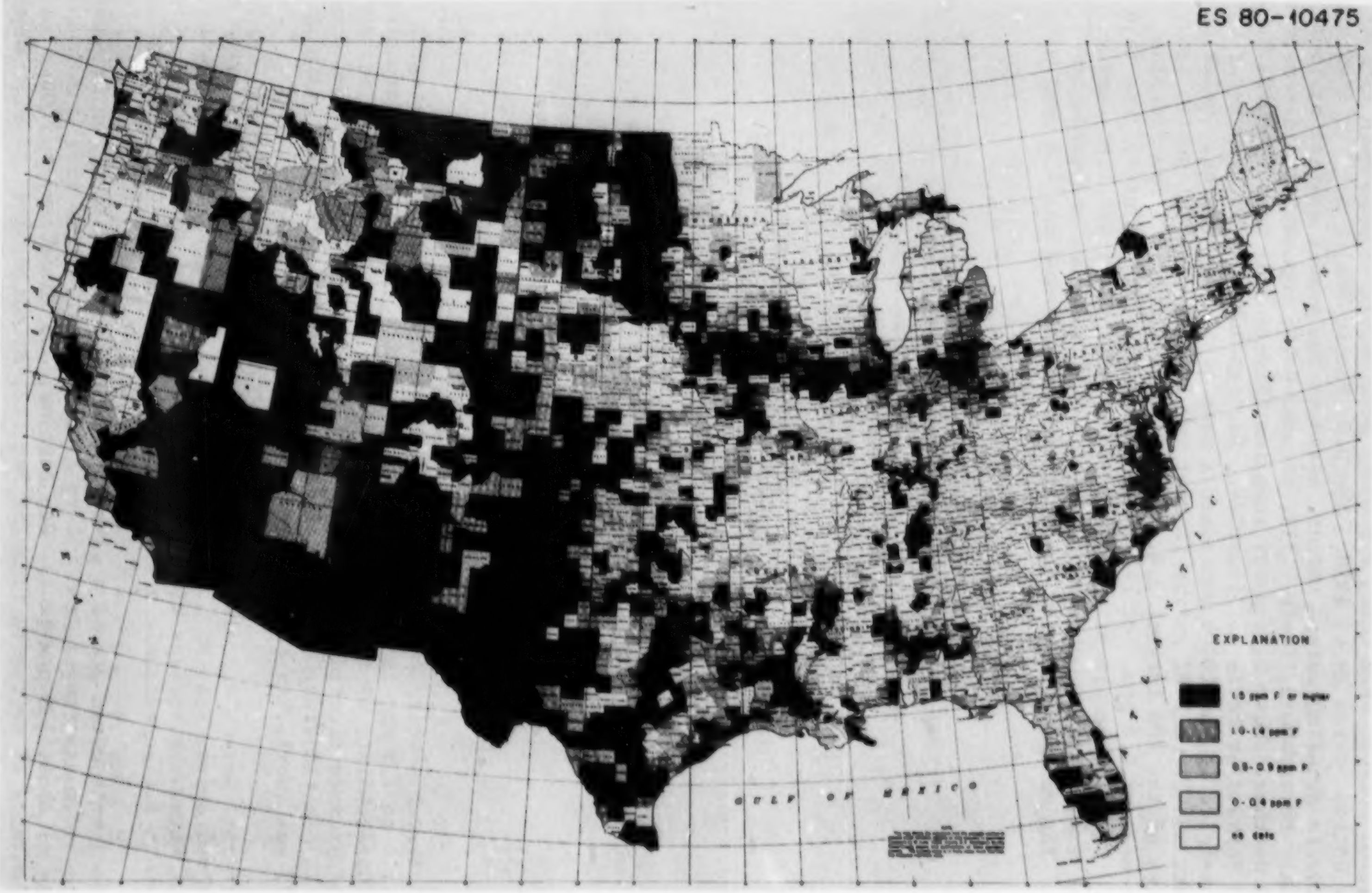
ES 80-10475
GULF OF MEXICO
EXPLANATION
1.5 ppm F or higher
1.0-1.4 ppm F
0.5-0.9 ppm F
0-0.4 ppm F
no data

Figure 7.3. Maximum fluoride content of U.S. waters (by counties). Source: Adapted from Fleischer and Robinson, 1963, Figure 3, p. 69.

appeared to be identical (Shishkina, 1966). There is no apparent difference in the fluoride content of bottom sediments of freshwater and saltwater lakes (Mun, Bazilevich, and Budeyeva, 1966).

Pore solutions extracted from sediments are enriched in fluoride. Interstitial waters in Georgia salt marshes show fluoride enrichment when compared with surface waters of the same chlorinity (Windom, 1971). The enrichment mechanism is thought to result from the uptake of fluoride from water by plants, followed by release of the fluoride to the sediments after plant death.

7.3.4 Distribution in Air

Fluorides enter the atmosphere as elemental fluorine, soluble gaseous fluorides (e.g., hydrogen fluoride), or soluble dusts (e.g., sodium fluoride) and insoluble dusts (e.g., cryolite) (Hodge and Smith, 1970). The fluoride concentration in ambient air depends on the amount of fluoride emitted, the distance from the source, the meteorological conditions, and the topography of the area (Davis, 1972). Total U.S. atmospheric fluoride emissions were about 150,500 metric tons per year as of 1970 (Smith and Hodge, 1978).

In rural areas where no industrial contamination exists, the atmospheric fluoride concentration is very low (fractions of a part per billion) (Hodge and Smith, 1972); urban air normally contains from less than 0.1 to 2.4 ppb (Tables 7.12 and 7.13) (Hodge and Smith, 1970). During 1966 to 1968, the National Air Surveillance Network collected over 11,300 24-hr samples of suspended particulates at urban and nonurban sites (Table 7.14). Of all urban measurements, 88% were below the limit of detection (0.05 μg/m^3) while over 98% of nonurban samples were below the limit. No geographic pattern of fluoride occurrence could be established (Thompson, McMullen, and Morgan, 1971).

Due to the burning of coal and other fossil fuels, atmospheric fluoride usually increases during the winter months. For example, during one nonheating season the atmosphere of British cities contained an average of about 0.03 μg/m^3. The fluoride concentration during the heating season averaged about 0.05 μg/m^3 (Lee et al., 1974). A similar winter increase is found in National Air Sampling Network data for 1953 to 1957 (U.S. Department of Health, Education, and Welfare, 1958) and in a study by Schneider (1968).

Fluoride is often concentrated in atmospheres near industrial operations (Table 7.15). Before controls were instituted, an aluminum plant in Oregon once released an average of 3175.2 kg (7000 lb) of fluoride per day (Savara, Noyes, and Suher, 1954). Since that time, controls have greatly reduced emissions. Schneider (1968) measured airborne fluorides near industrialized Duisburg, Germany, and found that 90% of the samples contained 0.5 to 3.8 μg of fluoride per cubic meter, with a mean of 1.3 μg/m^3. Air near an aluminum reduction plant in Scotland contained 220 μg/m^3 (Agate et al., 1949). One mile from the factory the concentration dropped to 40 μg/m^3. Hluchan, Mayer, and Abel (1968) found an average of 140 μg/m^3 near

TABLE 7.12. FLUORIDE IN PARTICULATE MATTER COLLECTED FROM THE AIR OF U.S. CITIES

City	Average fluoride value (ppm)
Los Angeles	0.45
Los Angeles (1954)	1.10
Detroit	0.05
Philadelphia	0.29
Chicago	0.04
New York	0.25
Cincinnati	0.25
Kansas City	0.01
Portland	0.00
Atlanta	0.06
Houston	0.00
San Francisco	0.45
Minneapolis	0.07
Charleston	0.00
Louisville	0.00
Pasadena	0.50

Source: Adapted from Cholak, 1959, Table II, p. 502. Reprinted by permission of the publisher.

TABLE 7.13. THE OCCURRENCE OF FLUORIDE IN THE ATMOSPHERE OF SOME U.S. COMMUNITIES

Location	Fluoride (ppb)	
	Mean	Range
Cincinnati (1957)	0.35	0.04-1.20
New York	2.0	
Yonkers (summer)		0.2-0.4
Spokane	1.6[a]	
Portland, Oregon		0.6-0.8
Rural	0.3	
Salt Lake City		0.05-1.0
Logan, Utah	0.02	
Canada-Washington Border	0.3[a]	
San Francisco area		0.2-0.4

[a] Single determination.

Source: Adapted from Cholak, 1959, Table III, p. 502. Reprinted by permission of the publisher.

TABLE 7.14. FLUORIDE IN URBAN AND NONURBAN U.S. AIR, 1966-1968

Year	Number of stations	Number of samples	Number of samples with fluorine content in the ranges shown				Maximum fluoride content ($\mu g/m^3$)
			<0.05 $\mu g/m^3$	0.05-0.09 $\mu g/m^3$	0.10-0.99 $\mu g/m^3$	1.00 $\mu g/m^3$	
			Urban				
1966	100	2521	2161	152	206	2	1.89
1967	122	2967	2612	134	212	9	1.74
1968	147	3687	3287	103	290	7	1.65
1966-1968		9175	8060	389	708	18	
			Nonurban				
1966	29	711	687	24	0	0	0.09
1967	30	729	721	5	3	0	0.16
1968	29	724	724	0	0	0	<.05
1966-1968		2164	2132	29	3	0	

Source: Adapted from Thompson, McMullen, and Morgan, 1971, Table IV, p. 486. Reprinted by permission of the publisher.

TABLE 7.15. FLUORIDES IN AIR NEAR INDUSTRIAL OPERATIONS

Operation	Airborne fluoride
Chemical manufacturing including AlF_3, aluminum metal in or near Odda, Western Norway	Maximum daily concentrations 5-103 μg F/m^3, mean concentrations 2.6-15.7 μg F/m^3, summer 1972, within 5 km
Aluminum smelter on Tiwai Peninsula opposite Bluff, New Zealand	0.3-0.4 ppb, 4-km downwind
Aluminum works, lower Fricktal, Switzerland	0.012-0.185 mg accumulated per month per dm^2 of sampler filter surface. Amounts varied with distance from source and wind direction.
Aluminum reduction plants near Massena, New York	53 seven-day ambient air samples collected at six sites over a period of approximately 10 weeks, range 0.1-2.0 ppb
Aluminum reduction plant	Lime-impregnated paper samplers during a 12-month period at three sites prior to start-up showed 0.2-0.5 μg/dm^2 per day, mean 0.35 μg/dm^2 per day; 1.6-4.6 km north of plant. Samples at all sites increased to a maximum of 5 μg/dm^2 per day after completion of start-up. Background levels 5 km from plant
Brick works	Mean of 31 samples 500 m NE of plant, 6.4 μg F/m^3 Mean of 12 samples 1000 m NE-NNE of plant, 1.9 μg F/m^3 Mean of six samples 300 m S-SSE of plant, 1.4 μg F/m^3
Gaseous diffusion for separation of uranium isotopes	Total of 572 samples collected at four locations. Maximum concentrations at different sites, 4 ± 0.3 to 5 ± 0.4 ppb F; means at different sites, 0.8 ± 0.1 to 1.0 ± 0.2 ppb F
Glazed tile manufacturing	0.020-0.043 ppm in the environmental air
Phosphate fertilizer manufacturer, gypsum pond, Manatee County, Florida	Pond water, 2810-5150 mg F/liter, evolving up to 0.094 lb F/acre per day
Phosphoric acid manufacturing	20 kg F discharged daily to atmosphere; 10% as apatite, 90% in gaseous state as SiF_4 and HF. 0-10 μg/m^3 in an area of 15.2 km^2 over ten-year period
Steel manufacturing	0.211-1.012 μg F/m^3

Source: Adapted from Smith and Hodge, 1978, Table 10, p. 301. Reprinted by permission of the publisher.

an aluminum factory in Czechoslovakia. Averages up to 3.90 μg/m^3 were recorded near Jacksonville, Florida, phosphate plants (Sheehy et al., 1963). Before these plants began operations, averages up to only 1.79 μg/m^3 were recorded.

The fluoride released during aluminum production is primarily in particulate form. Total fluoride emitted by a Maryland aluminum plant is composed of 64% particulate, 23% fluoride attached to particulate, and 13% free gaseous fluoride (Israel, 1974). A similar distribution was reported by Okita et al. (1974).

Emissions of fluorides in other selected industrial operations are discussed by Kaltreider et al. (1972), Krechniak (1969), Leidel et al. (1967), Luxon (1963), and Smith and Hodge (1978).

Attention has recently been focused on the concentrations of fluorocarbons present in the atmosphere. Important among these are Fluorocarbon 11 ($CFCl_3$) and Fluorocarbon 12 (CF_2Cl_2). Fluorocarbon 11 is used mainly as an aerosol propellant and a blowing agent for foam products. Fluorocarbon 12 is used in refrigeration and cooling systems (Anonymous, 1975*a*). Both of these compounds are found in concentrations of 0.05 to 0.1 ppb in air of remote areas and up to several tenths parts per billion in urban areas (Howard and Hanchett, 1975). Approximately 250 × 10^6 kg (551 × 10^6 lb) of fluorocarbons was released to the atmosphere from the United States in 1972 (Figure 7.4), and losses from non-U.S. sources are believed to be of similar magnitude.

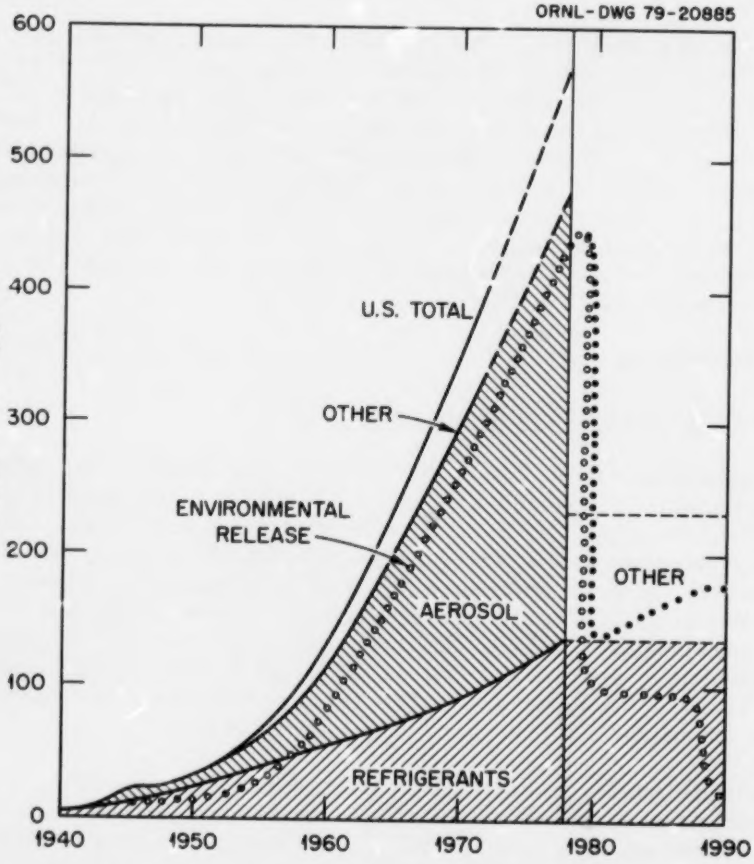

Figure 7.4. U.S. production and environmental release of fluorocarbons. Data to 1972 based on known production information. Trends were extrapolated to 1978, and then release calculations were made on the basis of the following assumptions: (1) zero production after 1978 (ooooo) and (2) no aerosol propellant use, with production for refrigerant and other uses remaining equal to the 1978 levels (•••••). Source: Adapted from Howard and Hanchett, 1975, Science, Vol. 189, 18 July 1975, Figure 1, p. 217. Copyright 1975 by the American Association for the Advancement of Science. Reprinted by permission of the publisher.

The high stability of fluorocarbons released to the environment allows them to diffuse to the upper atmosphere where they may be involved in the destruction of stratospheric ozone (O_3). In laboratory studies, Fluorocarbon 11 and Fluorocarbon 12 decompose in the presence of high energy ultraviolet radiation (as in sunlight) to yield intermediate carbene and chlorine atoms. The liberated chlorine atoms catalyze reactions that destroy O_3 (Anonymous, 1975*b*; Cicerone, Stolarski, and Walters, 1974; Cicerone, Walters, and Stolarski, 1975; Molina and Rowland, 1974). It has not been conclusively determined to what extent these reactions occur in the stratosphere (Interagency Task Force on Inadvertent Modification of the Stratosphere, 1975; Interdepartmental Committee for Atmospheric Sciences, 1975). If ozone is destroyed faster than it is formed by natural reactions, increased radiation will be allowed to reach earth, resulting in a health hazard.

Due to the potential health risk, the nonessential use of fluorocarbons in aerosol sprays was banned in the United States in 1978. Even with this curtailment, however, about 100×10^6 kg (220×10^6 lb) will be emitted annually during the next ten years (Figure 7.4). Furthermore, to be effective, a worldwide fluorocarbon ban must be implemented. Howard and Hanchett (1975) concluded that "restrictions of fluorocarbon production and use in the United States would only partly reduce, rather than end, destruction of stratospheric O_3, if, in fact, the chlorine-catalyzed O_3 destruction due to fluorocarbon compounds can exceed all natural sinks of stratospheric O_3."

7.4 ENVIRONMENTAL FATE

7.4.1 Mobility and Persistence in Soils

In alkaline soils, fluoride usually precipitates as calcium fluoride or forms soluble compounds with group I cations and magnesium (Mg), calcium (Ca), strontium (Sr), or barium (Ba) (Murrmann and Koutz, 1972). Soluble fluoride compounds are almost completely fixed in soils of pH 6.5 or above if sufficient calcium carbonate ($CaCO_3$) is available (Brewer, 1966). In situations where liming is not desirable, gypsum ($CaSO_4$) or some other neutral calcium salt will accomplish the same result. If adequate calcium is not present, fluoride may be fixed as aluminum silicofluoride (MacIntire et al., 1949). Sandy acidic soils favor development of soluble forms of fluoride (Shacklette, Boerngen, and Keith, 1974).

Fluoride tends to persist in most soils; it is strongly absorbed by soil colloids and cannot be displaced by common anions such as chloride or sulfate (Murrmann and Koutz, 1972). MacIntire, Sterges, and Shaw (1955) studied the retention of fluoride by four acidic soils over a four-year period (Table 7.16). The retention of fluoride was proportional to soil aluminum content; it was immobilized as the insoluble aluminum silicofluoride. Inputs of 2237 kg (4932 lb) of CaF_2 to phosphatic soils over a ten-year period resulted in leaching losses of only 2.5% of the added fluoride (annual rainfall of 129.5 cm, or 51 in.). Only after seven years did drainage waters contain as much as 1 ppm fluoride.

TABLE 7.16. FLUORIDE RETENTION FROM 200- AND 800-lb APPLICATIONS OF HYDROFLUORIC ACID IN FOUR SOILS

HF addition		Annual outgo of fluorine (lb per 2,000,000 lb of soil)									Final pH[c]
Rate[a]	Depth	1st	2nd	3rd	4th	Total	Increase (lb)	Increase (%)[b]	Retention (lb)	Retention (%)[b]	
					Hartsells sandy loam						
None		1.1	0.7	0.8	0.5	3.1					4.1
200	To surface	1.1	1.0	1.6	1.2	4.9	1.8	0.9	198.2	99.1	4.2
200	In full depth	4.5	2.3	4.0	3.7	14.5	11.4	5.7	188.6	94.3	4.1
800	To surface	3.5	4.4	12.4	16.6	36.9	33.8	4.2	766.2	95.8	4.3
800	In full depth	79.4	17.2	28.2	23.4	148.2	145.1	18.1	654.9	81.9	4.4
					Clarksville silt loam						
None		1.2	0.8	0.9	0.5	3.4					4.5
200	To surface	3.8	1.9	1.3	0.6	7.6	4.2	2.1	195.8	97.9	4.8
200	In full depth	7.3	3.1	2.7	2.3	15.4	12.0	6.0	188.0	94.0	4.6
800	To surface	126.3	23.9	23.6	17.5	191.3	187.9	23.5	612.1	76.5	4.8
800	In full depth	139.6	20.3	20.0	16.6	196.5	193.1	24.1	606.9	75.8	4.9
					Maury silt loam						
None		3.5	1.1	1.7	0.7	7.0					5.2
200	To surface	2.5	1.2	3.6	0.8	8.1	1.1	0.6	198.9	99.6	5.2
200	In full depth	5.0	2.0	2.4	1.1	10.5	3.5	1.8	196.5	98.3	5.2
800	To surface	3.0	2.0	4.5	0.8	10.3	3.3	0.4	796.7	99.6	5.0
800	In full depth	10.0	4.3	4.6	3.6	22.5	15.5	1.9	784.5	98.1	5.1
					Baxter silt loam						
None		4.9	1.3	1.7	0.8	8.7					4.9
200	To surface	6.6	4.4	3.6	2.9	17.5	8.8	4.4	191.2	95.6	4.8
200	In full depth	7.7	4.1	5.6	4.3	21.7	13.0	6.5	187.0	93.5	4.6
800	To surface	48.0	15.9	19.8	17.9	101.6	92.9	11.6	707.1	88.4	4.5
800	In full depth	73.1	31.1	27.6	26.7	158.5	149.8	18.7	650.2	81.3	4.5

[a] Pounds of fluorine per 2,000,000 pounds of soil.

[b] Respective to 200- and 800-lb additions of fluorine.

[c] Initial pH values: Hartsells, 5.40; Clarksville, 5.6; Maury, 5.3; and Baxter, 5.2. Rainwaters per annum for the four successive years were 57.5, 39.9, 44.0, and 40.7 in. respectively. Drainage waters were in range of 45% to 50% of precipitation.

Source: Adapted from MacIntire, Sterges, and Shaw, 1955, Table III, p. 780. Reprinted by permission of the publisher.

The amount of fluoride removed from soils by seepage water is small. Oelschlager (1971) collected groundwater samples from 30 widely separated forest and agricultural areas in Germany. Fluoride removed in seepage varied from 20 to 400 g/ha (0.02 to 0.4 lb/acre), or about 0.5% to 6.0% of the yearly added increment.

7.4.2 Mobility and Persistence in Water

In water, fluoride exists in both dissolved and undissolved forms. The undissolved fluoride, transported in or on clay minerals suspended in the water is eventually removed by sedimentation (Hamilton, 1974). Dissolved fluorides are usually present as the fluoride ion F^-, particularly in dilute solutions and at neutral pH (Borei, as cited in Largent, 1970). As pH increases, the proportion of F^- decreases while HF_2^- and undissociated HF increase. Dissolved fluorides readily precipitate as sediments in waters high in calcium. In marine waters, about 4×10^{11} g of fluoride per year are removed by incorporation into calcium carbonates (Table 7.17). Incorporation into calcium phosphates is apparently the second most important removal process. Assuming that the removal of fluoride from seawater

TABLE 7.17. RATES OF REMOVAL OF FLUORIDE FROM SEAWATER DUE TO INCORPORATION INTO VARIOUS MINERALS

Mineral	Fluoride removal rate (g/year)
Calcium carbonate	4.0×10^{11}
Calcium phosphates	$\leq 2.6 \times 10^{11}$
Authigenic montmorillonites	$\leq 1 \times 10^{11}$
Opal	1.5×10^{10}
Phillipsite	$\leq 2.8 \times 10^{9}$
Glauconite	$\leq 1.4 \times 10^{9}$
Ferromanganese nodules	2.3×10^{8}

Source: Adapted from Carpenter, 1969, Table 3, p. 1160. Reprinted by permission of the publisher.

by other minerals is negligible, removal by calcium carbonate and calcium phosphate amounts to about 4×10^{11} to 6.6×10^{11} g/year. Based on an oceanic volume of 1.37×10^{21} liters and an average fluoride content of 1.3 mg of fluoride per liter, the residence time of fluoride in the ocean is computed as 2 to 3 million years (Carpenter, 1969).

7.4.3 Mobility and Persistence in Air

Most volatile inorganic fluoride compounds hydrolyze in contact with water vapor in the air and are converted to less volatile compounds. Among the inorganic fluorides converted are silicon tetrafluoride, halogen fluorides, uranium hexafluoride, sulfur tetrafluoride, and disulfur difluoride. The resulting compounds are removed from the atmosphere by condensation or nucleation processes (National Academy of Sciences, 1971).

Particulate fluorides of industrial origin are usually stable compounds and do not readily hydrolyze. Among these are cryolite, calcium fluoride, fluorapatite, aluminum fluoride, sodium fluorosilicate, and sodium fluoride. All but sodium fluoride have low solubility in water and return to land as dusts. Other fluorides that do not hydrolyze readily (e.g., some of the fluorocarbons) may persist in the atmosphere until they are decomposed by radiation (National Academy of Sciences, 1971).

7.5 WASTE MANAGEMENT

Several waste treatment processes are currently used to remove fluoride from process waste streams. These processes and their effectiveness are described in Table 7.18. Usually, chemicals are added to the waste streams which result in the formation of fluoride precipitates or the adsorption of fluorides on resultant precipitates. The process is usually accomplished in two stages. Most of the fluoride is removed (leaving 10 to 20 mg/liter) by preliminary treatment with lime or other calcium

TABLE 7.18. FLUORIDE WASTE TREATMENT PROCESSES

Process	Initial fluoride concentration (mg/liter)	Final fluoride concentration (mg/liter)	Current application
Lime addition		10	Industrial
	1000-3000	20	Industrial
	1000-3000	7-8[a]	Industrial
	500-1000	20-40	Industrial
Alum coagulation	3.6	0.6-1.5	Municipal
Hydroxylapatite beds			
Synthetic	12-13	0.5-0.7	Municipal
	10	1.6	Municipal
Bone char	6.5	1.5	Municipal
	9-12	0.6	Municipal
Alumina contact beds	8	1	Municipal
	9	1.3	Industrial (lab scale)
	20-40	2-3	Industrial

[a] After 24-hr settling.

Source: Adapted from Watson, 1973, Table 36, p. 179. Reprinted by permission of the publisher.

compounds (Staebler, 1974, 1975; Watson, 1973; Zabban and Jewett, 1967). Further reductions (down to about 1 mg/liter) are affected by the addition of a second chemical such as magnesium hydroxide or aluminum sulfate (Miller, 1974; Skripach et al., 1970; Watson, 1973). The fluoride sludge resulting from the operations is normally disposed of as landfill (Ottinger et al., 1973).

Other techniques involve passage of gaseous- or liquid-fluoride wastes through a contact bed or slurry. The fluoride is removed by reaction with the bed matrix. Bed media include hydroxyapatite, ion exchange resins, activated alumina, iron, and charcoal (Kelly, 1974; Navratil, 1968; Ottinger et al., 1973; Reiter, 1972; Skripach et al., 1970; Watson, 1973).

SECTION 7

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SECTION 8

ENVIRONMENTAL INTERACTIONS AND THEIR CONSEQUENCES

8.1 SUMMARY

Fluoride cycles naturally among the lithosphere, atmosphere, hydrosphere, and biosphere. Man's activities, however, have resulted in new routes of fluoride transfer as well as additional flow through existing paths.

Food and water normally provide nearly all man's fluoride intake, and most foods and beverages contain fluoride in at least minute amounts. Certain foods and food products, such as seafoods, bonemeal, and tea, contain higher than normal levels. With these exceptions, food averages 0.1 to 0.3 ppm fluoride; consequently, dietary intake of fluoride is small and relatively constant, usually ranging from 0.20 to 0.80 mg/day in the United States.

A slow but steady accumulation of fluoride occurs in most animals. The increase is restricted almost entirely, however, to the skeletal tissues. Biomagnification of fluoride occurs at the lower end of the food chain; however, because fluoride is largely localized in skeletal tissues the uptake of fluoride in humans or other animals near the top of the food chain is not materially increased.

8.2 ENVIRONMENTAL CYCLING OF FLUORIDE

The environmental cycle for fluoride is presented in different ways in Figures 8.1 and 8.2. In the natural cycle, fluorides move from the

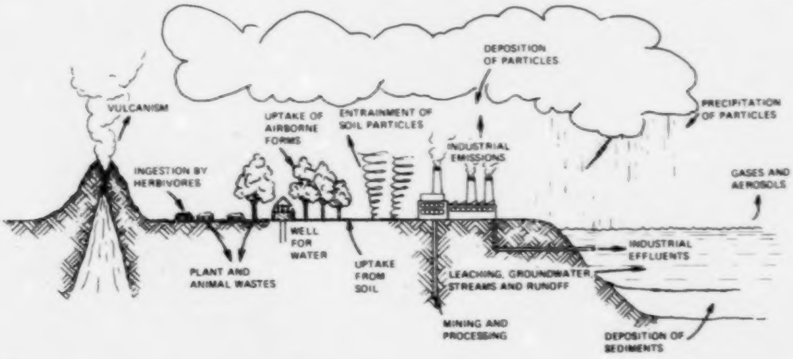
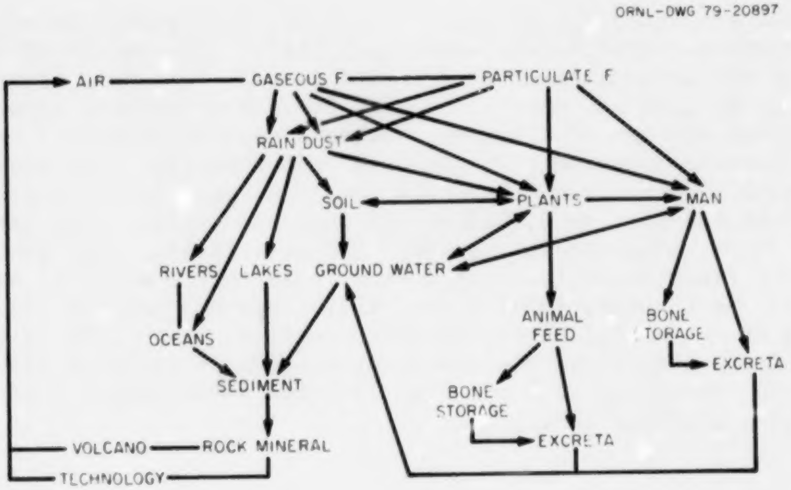

DEPOSITION OF PARTICLES
VULCANISM
UPTAKE OF AIRBORNE FORMS
ENTRAINMENT OF SOIL PARTICLES
INDUSTRIAL EMISSIONS
PRECIPITATION OF PARTICLES
INGESTION BY HERBIVORES
GASES AND AEROSOLS
WELL FOR WATER
INDUSTRIAL EFFLUENTS
PLANT AND ANIMAL WASTES
UPTAKE FROM SOIL
LEACHING, GROUNDWATER, STREAMS AND RUNOFF
MINING AND PROCESSING
DEPOSITION OF SEDIMENTS

Figure 8.1. Environmental transfer of fluoride. Source: Reprinted from Fluorides, Publ. 1922, p. 30, with the permission of the National Academy of Sciences, Washington, D.C.


ORNL-DWG 79-20897
AIR
GASEOUS F
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RAIN DUST
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PLANTS
MAN
RIVERS
LAKES
GROUND WATER
OCEANS
SEDIMENT
ANIMAL FEED
BONE STORAGE
EXCRETA
BONE STORAGE
EXCRETA
VOLCANO
ROCK MINERAL
TECHNOLOGY

Figure 8.2. Dispersion of fluoride in the biosphere. Source: Hodge and Smith, 1972, Figure 1, p. 164. Reprinted by permission of the publisher.

lithosphere to the atmosphere by way of volcanism or entrainment of soil particles. Routes from the hydrosphere to the atmosphere include vaporization and aerosol formation. Atmospheric fluoride returns to the hydrosphere and lithosphere in precipitation or by deposition of particulates. Fluoride also travels from the lithosphere to the hydrosphere by leaching and erosion and returns by sedimentation (National Academy of Sciences, 1971).

The biosphere serves as an intermediate repository for part of the fluoride that passes from the atmosphere to the lithosphere. Fluoride in vegetation is returned directly to the lithosphere in plant wastes or enters the food chain and is returned in animal wastes.

Man's activities result in new routes of fluoride transfer and additional flow in the existing ones. For example, industrial processing involving fluorides results in a direct atmospheric emission, and lithospheric fluoride is increased by industrial effluents and waste waters, resulting in an increase in the fluoride burden of other phases of the environment (National Academy of Sciences, 1971).

8.3 FLUORIDE IN FOODS

The principal sources of fluoride intake for humans are food and water. Factors affecting the fluoride content of foods include the locality in which the food is given, the amount of fertilization and spraying received, the type of food processing, and whether fluoridated water is used in preparation (McClure, 1949; Myers, 1978; Waldbott, 1963). These factors change from place to place, resulting in varying amounts of fluoride in foods produced in different localities. The extent of these effects on the concentration of fluoride in U.S. foods can be seen in Table 8.1. In general, recent analyses of edible red meat show only a few parts per million of fluoride. Cereals usually contain less than 1 ppm, but samples containing 20 ppm have been reported. Vegetables and tubers usually range from 0.1 to 0.4 ppm; hen eggs, 0.1 to 0.6 ppm; cow milk, 0.1 to 0.6 ppm; nuts, 0.1 to 1.5 ppm; and fruits, less than 1 ppm (Cholak, 1959; Hodge and Smith, 1972; McClure, 1949). Average concentrations of fluoride in foodstuffs from India appear somewhat higher (Lakdawala and Punekar, 1973; Nanda, 1972; Sengupta and Pal, 1971). Beverages, with the exception of tea which contains up to 0.52 μg of fluoride per cup (Cook, 1969), are not important sources of fluoride (Table 8.1). A few wines contain up to 6 ppm fluoride, but most contain less than 1 ppm (Hodge and Smith, 1970).

TABLE 8.1. FLUORIDE CONTENT OF VARIOUS FOODS AND BEVERAGES

Food or beverage	Fluoride content (mg/kg)	Mean fluoride content (mg/kg)
	Cereals	
Wheat	0.1-1.2[a]	
	0.26-1.3[a]	
	0.1-4.0	
	0.18-3.15	0.93
Rye	0.42-7.1	1.53
	0.30	
Barley	0.4-4.8	1.18
Oat	0.18-0.78	0.45
Rice	21.2	
Polished rice	11.4	
	Vegetables	
Potato	0.12-2.9	0.56
	0.07-6.4	
	0.1-1.1	
Carrot	0.19-0.60	0.28
	0.22-2.00	
Lettuce	0.18-0.42	0.28
Spinach	0.44-6.4	2.82
	0.1-20.3	
	0.26	
	13.6[a]	
Bean	0.11-0.15	
Parsley	0.35	
	Fruits	
Citrus fruits	0.03-0.36	
	0.07-0.36	
Other fruits	0.11-1.32	
	0.35-2.1	
Dried plums	0.08	
Apples	0.61-0.64	
Pears	1.07-1.11	
Oranges	0.31-0.41	
	Beverages, spices, and products of vegetable origin	
Tea, leaf	3.2-398.8	
Georgian	120	
Indian	89	
Ceylon	82.0	
Drink, 1 cup	0.5	
Coffee	0.2-1.6	
Wine and beer	0.07-0.24[b]	
White wine	0.20-1.7[b]	
Red wine	0.084-0.94[b]	
Nuts	0.3-1.5	

TABLE 8.1 (continued)

Food or beverage	Fluoride content (mg/kg)	Mean fluoride content (mg/kg)
Crude sea salt (Indian)	35-55	
Crude sea salt (Greek)	18-20	
Purified sea salt (Greek)	4	
Meats, dairy products, and eggs		
Broiler	1.4	
Mutton	1.20	
Beef	0.20-2.00	
Pork	0.20-3.30	
Liver (beef)	5.20-5.80[a]	
Kidney (beef)	6.90-10.10[a]	
Heart (beef)	2.30-2.70[a]	
Liver (pig)	0.29	
Eggs	1.2	
Milk	0.20-0.40	
	0.21-0.27	
	0.09-0.32[b]	
	0.09-0.36[b]	
	0.06-0.10[b]	
	0.07-0.22[b]	
Cheese	1.62	
	0.16-1.32	
Butter	1.50	
Kefir	0.06-0.10[b]	
Fish, fish products, and crustaceans		
Codfish, fresh	3.73-4.34	
	1.9-3.2	
Fillet	2.7	
Haddock (fillet)	5.06	
Herring (cleaned)	28.5	
Herring (fillet)	5.36	
Herring (Atlantic)	2.3	
Tuna	3.52-4.00	
Anchovy (in oil)	2.83-3.18	
Sardine (in oil)	5.0	
	4.12-4.40	
Mackerel	3.19-3.89	
Fish scales	5.0-10.0	
Clam	1.34-1.83	
Shrimp	2.27-2.53	
Sea urchin	2.87-3.20	
Lobster	1.99-2.18	
Fish protein concentrate	20-760[a]	

[a] mg/kg dry weight.
[b] mg/liter.

Source: Adapted from Kumpulainen and Koivistoinen, 1977, Tables V-IX, pp. 43-47. Data collected from various sources. Reprinted by permission of the publisher.

Certain foods and food products tend to be enriched in fluoride. Seafoods, particularly fish and crustaceans, contain high concentrations of fluoride due to constant exposure to seawater (Cholak, 1959). Animal products such as bonemeal and gelatin are rich in fluoride because they are made from skeletons and hides, which accumulate fluoride (Section 5). Fish protein concentrate contains as much as 761.0 ± 0.1 ppm fluoride (Ke, Power, and Regier, 1970); however, studies by Zipkin, Zucas, and Stillings (1970) indicated that the fluoride in fish protein concentrate is only one-fourth to one-half as available for deposition in various hard tissues and the whole bodies of rats as is sodium fluoride.

Commercially prepared baby foods may have relatively high concentrations of fluoride. Some samples of Pablum contained 4 to 12 ppm, and orange juice and formula contained up to 1.0 ppm (Farkas and Farkas, 1974; Tinanoff and Mueller, 1978; Wiatrowski et al., 1975). Ericsson and Ribelius (1971) reported formula preparations with up to 53 times the fluoride levels of mother's milk. Ham and Smith (1950) calculated that a four-month-old infant received 0.46 mg of dietary fluoride per day. Wiatrowski et al. (1975) analyzed many baby foods and concluded that dietary intake of fluoride ranged from 0.32 mg/day for one- to four-week-old infants to 1.23 mg/day for infants four to six months of age. These intakes may be compared with recommended fluoride intakes of 0.5 mg/day for children from infancy to three years of age that live in areas where water is not fluoridated (Committee on Nutrition, 1972; McClure, 1970). Adair and Wei (1978) also concluded that infants fed with commercially prepared formulas may be receiving fluoride in excess of recommended optimal daily doses.

Foods characteristically high in fluoride include seafood, bone products, and tea. Foods with typically low fluoride content include most fruits and vegetables. With these exceptions, foods produced far from point sources of fluoride usually average 0.1 to 0.3 ppm fluoride (Cholak, 1959). Foods grown near fluoride-emitting factories often contain higher than normal concentrations of fluoride. For example, unwashed winter cabbage grown near point sources in the Stoke-on-Trent area (England) during 1965-1966 averaged 12.7 ppm fluoride (dry-weight basis), and some unwashed summer lettuce from the same area contained almost twice this concentration of fluoride (Jones, Harries, and Martin, 1971).

Many foods receive additional fluorides during processing, primarily due to the use of fluoridated water (Table 8.2). Uptake of cooking-water fluoride by vegetables is proportional to the fluoride content of water

TABLE 8.2. FLUORIDE CONTENT OF VARIOUS FOODS AND BEVERAGES PROCESSED IN EITHER FLUORIDATED OR UNFLUORIDATED WATER

Food or beverage	Fluoride content (ppm)				Difference (ppm)
	Unfluoridated water		Fluoridated water		
	Liquid	Solid	Liquid	Solid	
Pork and beans		0.27		0.77	+0.50
Tomato soup	0.04		0.38		+0.34
Ginger ale	0.02		0.77		+0.75
Beer	0.30		0.68		+0.38
Mixed vegetables	0.30	0.37	1.03	1.05	+0.68[a]
Green beans	0.14	0.20	0.71	0.89	+0.69[a]
Whole potatoes	0.13	0.38	0.87	0.76	+0.38[a]
Diced carrots	0.30	0.19	0.55	0.61	+0.42[a]
Kernel corn	0.10	0.20	0.48	0.56	+0.36[a]
Green peas	0.15	0.10			
Wax beans			0.49	0.60	
			0.77	0.73[b]	

[a] Solid

[b] This sample of wax bean was sent as an unfluoridated "control" sample. However, subsequent investigation revealed that the product had been processed in a cannery using well water containing 1.2 ppm F^-.

Source: Adapted from Marier and Rose, 1966, Food Technology/Journal of Food Science, Vol. 31, pp. 941-946. Copyright by Institute of Food Technologists. Reprinted by permission of the publisher.

over the concentration range of 1 to 5 ppm, but frozen vegetables absorb more fluoride than fresh vegetables, and those cooked in a saucepan take up more fluoride than those cooked in a pressure cooker (Martin, 1951). In general, water containing 1 ppm fluoride will increase the fluoride content of processed foods and beverages by 0.5 ppm or more (Auermann, 1973; Marier and Rose, 1966). Thus, food processed with fluoridated water may contain 0.6 to 1.0 ppm fluoride rather than the "normal" 0.2 to 0.3 ppm, providing an increase of about 0.5 mg/day in the diet.

Despite the widespread occurrence of fluoride in foods, human intake through diet is usually small and relatively constant, depending on consumer age and environment (Table 8.3). Daily dietary intake, including drinking water, has been established for many areas of the world (Tables 8.4-8.6). Daily intake is usually at least 0.2 mg, and in diets rich in fish, tea, or other fluoride-containing liquids, it can be as high as 5 mg or more (Auermann, 1973; Elliot and Smith, 1960; Prival and Fisher, 1974). Generally, men consume about 1.8 mg of dietary fluoride per day, housewives about 1.3 mg, and children 5 to 14 years old, 0.6 mg (Cholak, 1960). Young adult males eat more heartily than other subgroups of the population and consequently ingest more fluoride. In a 1967-1968 study, the typical young adult U.S. male consumed 2.1 to 2.4 mg of fluoride per day (San Filippo and Battistone, 1971). However, contemporary U.S. army field rations contained less than half the fluoride in the typical civilian diet (San Filippo, Battistone, and Chandler, 1972).

TABLE 8.3. ESTIMATED DAILY FLUORIDE INTAKE FROM FOOD AND DRINKING WATER[a]

Age (years)	Body weight (kg)	Fluoride intake (mg)			Total fluoride (mg/kg body wt)
		From drinking water	From food	Total from food and drinking water	
1-3	8-16	0.390-0.560	0.027-0.265	0.417-0.825	0.026-0.103
4-6	13-24	0.520-0.745	0.036-0.360	0.556-1.11	0.023-0.085
7-9	16-35	0.650-0.930	0.045-0.450	0.695-1.380	0.020-0.068
10-12	25-54	0.810-1.17	0.056-0.560	0.866-1.73	0.016-0.069

[a] Food (dry substance) contained 0.1 to 1.0 mg of fluoride per liter; drinking water contained 1 mg of fluoride per liter.

Source: Adapted from Maier, 1971, Table 2, p. 390.

8.4 BIOACCUMULATION IN FOOD CHAINS

Ingestion of fluoride-containing materials by animals results in a small but steady accumulation of fluoride within the body (National Academy of Sciences, 1971). The fluoride accumulates almost exclusively in the skeletal system (Hodge and Smith, 1970). Although accumulation occurs, the overall retention of daily ingested fluoride is low due to rapid elimination of fluoride by the body (Hodge and Smith, 1970) (Section 5).

TABLE 8.4. DAILY DIETARY FLUORIDE INTAKE FOR VARIOUS COUNTRIES

Country	Fluoride in food (mg)	Fluoride in water (ppm)
Canada	0.18-0.3	0.1
England	0.3-0.5	Trace
Japan	0.47-2.66[a]	0.01-0.08[b]
Newfoundland	2.74[c]	Trace
Norway	0.22-0.31	0.01-0.07
Russia	0.6-1.2	0.2-0.3
Sweden	0.9	
Switzerland	0.5[d]	
United States	0.2-0.3[d]	
	0.34-0.80	0.1

[a] Including 0.07-0.86 mg from green tea.

[b] Milligrams of fluoride ingested.

[c] Including 1 mg from tea.

[d] Exclusive of that in drinking water.

Source: Adapted from Hodge and Smith, 1972, Table 2, p. 165. Reprinted by permission of the publisher.

TABLE 8.5. ESTIMATES OF TOTAL DAILY FLUORIDE INTAKE IN THE UNITED STATES, THE UNITED KINGDOM, RUSSIA, AND JAPAN

Country	Daily fluoride intake per person (mg)
	Food
United States[a]	0.2-0.3
	0.25-0.32
	0.45
	0.25-0.55[b]
	1.0-2.0[c]
United Kingdom	0.3-0.5
	0.6-1.8[d]
Russia	1.18
Japan	4.5
	Food and fluoridated drinking water
United States	2.5 (av 3.1)
	0.34-3.13
United Kingdom	1.2-3.2
	5.5
Japan	5.0

[a] Estimates by various researchers.

[b] One- to two-year-old children.

[c] Fluoridated water used in food preparation and preserving.

[d] Four beverages.

Source: Adapted from Jones, Harries, and Martin, 1971, Table II, p. 603. Reprinted by permission of the publisher.

TABLE 8.6. FLUORIDE IN DIETS IN NORTH AMERICA

Diet	Fluoride in food (mg/day)	Fluoride in liquid (mg/day)	Total fluoride (mg/day)
Three meals served to house staff at Minnesota general hospital (excluding water)[a]	0.27-0.32		
Daily intake of one person in Cincinnati measured over 20 weeks[a]	0.155 (av) 0.54 (max)	0.299 (av)[b] 0.75 (max)[b]	0.457 (av)
Three restaurant meals in southern Ontario[a]			0.18-0.3
Estimates from existing data on fluoride content of specific foods; assumed water contains 1 ppm fluoride	0.2-0.3	1.0-1.5[c]	1.2-1.8
Diets of three young women in Toronto avoiding high-fluoride foods such as fish and tea[a]	0.43-0.76	0.0-0.03	0.43-0.79
Analysis of market-basket samples			
Baltimore[d]			0.8
Dallas[e]			0.9
Atlanta[a]			2.1
Minneapolis[d]			2.3
Four market-basket samples in Baltimore (artificially fluoridated to approximately 1 ppm fluoride)	0.78-0.90	1.3-1.5	2.1-2.4
Calculated from diets of seven laboratory workers, assuming all food processed in water at 1 ppm fluoride and all drinking and cooking water at 1 ppm fluoride	1.0-2.0	1.0-3.2	1.9-5.0
Analyzed diets (designed for low calcium content) of ten patients in Hines, Illinois;[f] water fluoridated to 1 ppm	1.2-2.7 2.0 (av)	1.6-3.2 2.4 (av)	3.6-5.4 4.4 (av)
Calculated value for one meal including high-fluoride mineral water and fish			3.3

[a] Water low in fluoride (<0.2 ppm).

[b] Including tea.

[c] Includes water used for cooking as well as drinking.

[d] In Baltimore and Minneapolis, water was fluoridated to approximately 1 ppm fluoride.

[e] Reported fluoride levels in Dallas water vary from 0.2 to 0.7 ppm.

[f] These values are considered atypical by some authorities due to the probable substitution of fluoridated water for low-fluoride beverages normally consumed by these patients (Myers, 1978).

Source: Adapted from Prival and Fisher, 1974, Table 1, p. 30.

Some data exist on fluoride accumulation in plants and animals (Table 8.7; Sections 4 and 5) (Jones, Harries, and Martin, 1971; Martin and Jones, 1971; Oelschlaeger, Feyler, and Schwarz, 1972), but there is an absence of literature on the movement of fluoride in the natural food chain (Kay, Tourangeau, and Gordon, 1975). Carlson (1973) and Kay et al. (1975) reported that fluoride ingested by prey species accumulates in predators that consume the prey. It is assumed that the fluoride in predators results from consumption of prey skeletons. The retention of fluoride is not great, however, and animals grown for human consumption generally do not contain enough fluoride to significantly increase human uptake (National Academy of Sciences, 1971). Thus accumulation of fluoride through the food chain does not appear to be a serious problem under normal circumstances.

TABLE 8.7. BIOACCUMULATION OF FLUORIDE IN SELECTED PLANTS AND ANIMALS

Organism	Tissue	Fluoride content[a] (ppm)	Parameters of exposure
Lichens	Whole plant	475-990	Air, 1-8 km from source, 4 or 12 months
		134-190	Air, 15 km from source, 12 and 4 months
		70	Air, 40 km from source, 4 months, control
Mosses	Whole plant	140-600	Air, 1-4 km from source, 4 or 12 months
		78	Air, 15 km from source, 12 months
		20	Air, 40 km from source, 4 months, control
Vegetation (all types)		6.1-10.7	Control samples
		10-70	Samples 4-14 miles from source, 200,000+ acres
		100-1000+	Samples 2 miles or less from source, 7040 acres
Insects			All specimens exposed to fluoride in air and diets
Controls (all types)	Whole body	3.5-16.5	Captured 50 miles from source
Foliage feeders	Whole body	21.3-255	Captured within 1/2 mile of source
Cambial feeders	Whole body	8.5-52.5	Same
Predators	Whole body	6.1-170	Same; primary source of intake, insects in diet
Pollinators	Whole body	58-585	Same
Chipmunk	Femur	50-303 (108.7)	Controls
		200-13,333 (1000)	Adults from assorted locations in polluted area
Ground squirrel	Femur	35-142 (105)	Controls
		17-7168 (775)	From assorted locations in polluted area
Deer mouse	Femur	(106.5)	Controls
		28-6038 (1300)	From assorted locations in polluted area
Deer (two species)	Femur	86-635 (225)	Controls
		124-9400 (1500)	From assorted locations in polluted area
Vegetation (all types)		1.6-6.4 (3.9)	Controls
		6-456	From assorted locations in polluted area, 2 years (samples above 35 ppm all 2.5 km or less from source)
Deer mouse	Femur	(144)	Controls
		288-1995	From assorted locations in polluted area, 2 years
Shrew	Femur	(494)	Controls
		1315-10,500	From assorted locations in polluted area, 2 years
Sparrow	Bones	84-565	Controls
		1013-3527	Captured near factory (aluminum smelter)
Frog	Bones	392-1067	Controls
		852-7880	Captured near factory
Grass		10-30	Controls
		up to 1330	Growing near factory
Freshwater plants		(40.5)	Exposed to water of unknown fluoride content
Water hyacinth	Leaf	25	Growing naturally in water at 1 ppm fluoride
	Petiole	60	Same
Marine algae (several varieties)		2-22	Growing naturally in seawater at 1.08 ppm fluoride
Eel grass	Leaves	0.2	Kept in seawater at 1.05 ppm fluoride, 72 days
		Trace	Kept in seawater at 52 ppm fluoride, 72 days

TABLE 8.7 (continued)

Organism	Tissue	Fluoride content[a] (ppm)	Parameters of exposure
Algae (*Cladophora*)		3.2	Kept in seawater at 1.05 ppm fluoride, 72 days
		2.4	Kept in seawater at 52 ppm fluoride, 72 days
Sand shrimp	Total body	106	Kept in seawater at 1.05 ppm fluoride, 72 days
		3116	Kept in seawater at 52 ppm fluoride, 72 days
Mud crab	Total body	169.6	Kept in seawater at 1.05 ppm fluoride, 72 days
		1414	Kept in seawater at 52 ppm fluoride, 72 days
Prawn	Total body	374	Kept in seawater at 1.05 ppm fluoride, 72 days
		3248	Kept in seawater at 52 ppm fluoride, 72 days
Mullet	Total body	141.8	Kept in seawater at 1.05 ppm fluoride, 72 days
		7743	Kept in seawater at 52 ppm fluoride, 72 days
Oyster	Soft tissues	4-5	Kept in seawater at 0.5 ppm fluoride for up to 60 days
		13-18	Kept in seawater at 2.0 ppm fluoride for up to 60 days
		20-30	Kept in seawater at 8.0 ppm fluoride for up to 60 days
		50-100	Kept in seawater at 32 ppm fluoride for up to 35 days
Blue crab	Exoskeleton	298	Controls, kept in seawater at 0.5-1.5 ppm fluoride
	Gills	253	Same
	Muscle	10	Same
	Exoskeleton	800	Kept in seawater at 8 ppm fluoride for 30 days
	Muscle	21	Same
Brown trout	Bone	400-1700	Living in stream with natural fluoride content 1-14 ppm
Trout	Fillet	1.3	Exposure unknown: assumed to be control level
Ocean fish (various)	Soft tissues	3.5-5.7	Living in seawater, 0.7-1.4 ppm fluoride
	Skeletons	558-6820	Same
Clover	Leaf and stem	6-13	Controls
		8-17	100 ppm fluoride added to soil as NaF, KF, or cryolite
		17-53	600 ppm fluoride added to soil as NaF, KF, or cryolite
		62-233	1800 ppm fluoride added to soil as NaF, KF, or cryolite
Spinach	Leaf	5.3	Control
		15.5	280 ppm fluoride as NaF added to soil
		41.3	560 ppm fluoride as NaF added to soil
Winter rye	Leaf and stalk	6.0	Control
		67.0	500 ppm fluoride as NaF added to soil
		158	1000 ppm fluoride as NaF added to soil
		200	1500 ppm fluoride as NaF added to soil

[a] Figures in parentheses are average values, when available.

Source: Adapted from Groth III, 1975, Table 1, pp. 227-228. Data collected from several sources. Reprinted by permission of the publisher.

SECTION 8

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SECTION 9

ENVIRONMENTAL ASSESSMENT OF FLUORIDE

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9.1 INTRODUCTION

Voluminous literature has been accumulated and reviewed regarding fluorine and the biological aspects of its compounds. The literature is extensive beyond the possibility of complete coverage; therefore, this summary is one of judgment without pretense of completeness.

9.2 PROPERTIES AND ENVIRONMENTAL OCCURRENCES

Fluorine, a halogen, is a pale yellow acrid gas. It is a common element, widely distributed, constituting 0.06% to 0.09% of the earth's crust. Fluorine is highly reactive and rarely occurs in nature in the elemental form but combines chemically to form fluorides. It appears in detectable concentrations in most soils, rocks, waters, the atmosphere, vegetation, and animal tissues. The most common chemical forms are hydrogen fluoride, alkali fluorides, silicon tetrafluoride, sodium fluorosilicate, fluorocarbons, and the minerals cryolite, fluorapatite, and fluorspar.

Important naturally occurring fluoride sources include volcanic activity, erosion of rocks and soils, and geothermal waters. Fluoride occurs in a wide variety of minerals. Substantial quantities are present in certain mineral and rock formations.

Major sources of fluorides used in industry include fluorspar (fluorite), cryolite, and fluorapatite. The United States produces only part of the fluorides it uses (20% in 1970) and imports the balance primarily from Mexico, Spain, and Italy. If economical methods of recovering and recycling industrial waste fluorides could be developed, the United States could supply its own needs.

A variety of analytical methods are available for determining fluoride content of many substances. The widely used spectrophotometric procedures of the past have been largely superseded by the fluoride ion electrode method which is quicker and more convenient than other methods for most types of samples. Nevertheless, fluoride must first be separated from often relatively large amounts of extraneous material without significant loss of fluoride.

9.2.1 Soils

Soils may contain diverse fluoride forms in highly variable amounts. In undisturbed and unpolluted soils, the quantity present generally increases with depth. Analyses may vary from less than 10 ppm fluoride to

10,000 ppm fluoride. Average fluoride values include: igneous rocks, 625 ppm; sandstones, 270 ppm; shales, 740 ppm; limestone, 330 ppm; and soils, 200 to 300 ppm. Clays usually contain more than sandy soils. Fluorides tend to persist longest in soils with a high calcium or aluminum content. Fluorides in sandy or acid soils are generally more soluble and either leach out or are more available for take up by vegetation. Application of phosphate fertilizers, irrigation with water of high fluoride content, and certain industrial emissions may raise the fluoride content of surface soils. However, these fluorides often are, or rapidly become, relatively insoluble forms that are not readily available to plants growing on those soils.

9.2.2 Water

Fluorides are naturally occurring constituents of most waters. Uncontaminated surface waters (e.g., rivers and lakes) usually contain less than 0.3 ppm fluoride.

Groundwater sources such as springs and deep wells usually contain somewhat higher fluoride levels. Fluoride content of groundwater is at least partially dependent on the type of rocks the water flows over and the temperature of the water. Alkalic igneous rocks, dolomite, phosphorite, and volcanic gases are most likely to give water of high fluoride content. Climatic conditions such as amount of rainfall and evaporation rate also affect the fluoride content of groundwater. Warmwater sources (including geothermal wells) are much more likely to have an elevated fluoride level than are cold sources. Fluoride levels in warm sources usually range up to about 15 ppm fluoride, although a few higher levels have been reported.

Seawater usually averages about 1.4 to 1.5 ppm fluoride. Oceanic sediments average about 730 ppm fluoride. The Great Salt Lake in Utah averages about 14 ppm fluoride, but some analyses of lakes and potholes in Kenya, Africa, range up to several hundred ppm fluoride.

The U.S. Public Health Service has recommended that drinking water contain from 0.7 to 1.2 ppm fluoride (level dependent upon average ambient air temperature) as the most efficient and economical method of providing an optimal fluoride intake for development of caries-resistant teeth.

9.2.3 Air

Fluorides occur in the atmosphere either naturally or as industrial emissions. The fluorides may be either in gaseous or particulate (dust) forms.

Atmospheric fluoride levels are normally very low and are measured in fractions of a part per billion (ppb) (often less than 0.05 $\mu g/m^3$ ppb). Some industrial emissions, dust storms, escaping volcanic gases, or combustion products of winter heating may locally raise the above level by 8 to 20 times or more. Levels are dependent on amount of fluoride emitted, distance of sampling site from source(s), meteorological conditions, and area topography. Current estimates of total U.S. atmospheric fluoride emissions are greater than 155,000 tons per year.

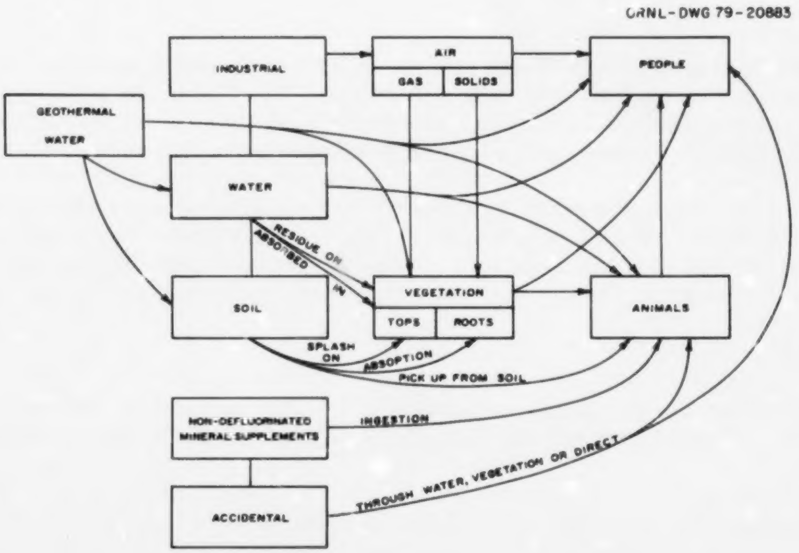
One major source of concern for the atmosphere appears to be related to the widespread release of certain fluorocarbons. Some of these compounds have been widely used as aerosol propellants and as refrigerant gases. The purported danger comes from the possible reaction of these products with the ozone layer in the upper atmosphere. Some reactions have been demonstrated in the laboratory. The ozone layer serves as a shield from dangerously high levels of ultraviolet radiation from the sun. Elemental fluorine is apparently not active in the reaction but is simply present in the compounds in question. Efforts are underway to drastically curtail the use and release of these fluorocarbons.

9.3 ENVIRONMENTAL INTERACTIONS

Fluorides naturally cycle among soils, water, air, and biological forms. Pathways that fluorides may take within the environment are numerous and complex. Figure 9.1 illustrates some of the possible pathways to plants, animals, and people. Man's activities, especially specific industrial operations, have increased the quantities of fluorides being cycled and the number of areas where the burden has been great enough to induce some detrimental effects on some ecosystems.

9.3.1 Soils

Fluorides tend to persist in most soils. They are strongly absorbed by soil colloids and combine with calcium or aluminum in alkaline soils


ORNL-DWG 79-20883
INDUSTRIAL
AIR
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PEOPLE
GEOTHERMAL WATER
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ROOTS
ANIMALS
SPLASH ON
ABSOPTION
PICK UP FROM SOIL
NON-DEFLUORINATED MINERAL SUPPLEMENTS
INGESTION
ACCIDENTAL
THROUGH WATER, VEGETATION OR DIRECT

Figure 9.1. Routes of fluorides in the environment. The multiple sources may ultimately supply beneficial amounts or excessive quantities to people.

to form only slightly soluble compounds that are stable and long retained under most conditions. Usually fluorides are not readily leached from soils.

9.3.2 Water

Fluorides are found in water in both dissolved and undissolved forms. Unless chemically altered, undissolved forms eventually settle out of the solution. The amount of fluorides maintained in the dissolved state depends on pH, temperature, and the presence of strongly associating ions such as magnesium, calcium, carbonates, and phosphates.

9.3.3 Air

Most volatile inorganic fluoride compounds react with water vapors to form less volatile compounds. These compounds are then removed from the atmosphere by condensation or nucleation. Stable atmospheric particulate compounds usually fall out as dust. Some of the fluorocarbons may persist in the atmosphere until they are decomposed.

9.3.4 Industrial Effluents

Steel and aluminum production, phosphate ore processing for fertilizers, production of phosphoric acid and a variety of other compounds, coal combustion, and glass and ceramic manufacturing are sources of industrial fluoride emissions. Welding and metal casting often use fluorides as fluxes, and these are often released into the atmosphere with little or no efforts made to capture them.

Industrial effluents containing excessive fluorides may have these removed by several methods. Gaseous wastes are usually washed so that the wastes are trapped in water. These wastes or water contaminated by unwanted fluorides are usually treated with appropriate chemicals to form precipitated forms, or the fluorides are absorbed in contact beds or slurries. Chemicals that may be used, depending on the individual circumstances, include: lime or other calcium compounds, some magnesium or aluminum compounds, ion exchange resins, or charcoal. The resulting sludges of fluoride waste can be filtered and disposed of.

9.3.5 General Biological Aspects

The mode of action and effects of fluorides on biological systems are important and complex. In optimal amounts fluorides are beneficial to some vegetation, some animals, and man. In excessive amounts they are detrimental.

The mechanisms by which fluorides function are not totally understood. Effects of fluoride and the responses it elicits have been studied on whole organisms and on various components such as cells, tissues, and organs. At optimal concentrations, fluorides are reported to activate or enhance a variety of physiological processes including reactions of some enzymes and growth stimulation in vegetation. Excessive levels, however, induce adverse physiological responses such as inhibition of some enzyme activities.

Physiological effects of fluoride on isolated cells and tissues may be very different from its effects on the total organism. Extrapolation of experimental laboratory test results to living systems should be done cautiously because of these differences. Often, fluoride concentrations used in laboratory tests far exceed those found in the environment. Because of the many factors and variables usually associated with fluoride toxicosis, extrapolation of confined experimental data to practical situations is often difficult. Likewise, it is often difficult to scientifically prove that fluorides are responsible for some of the adverse and detrimental effects sometimes attributed to them.

9.4 MICROORGANISMS

Many bacteria, yeast, fungi, algae, protozoa, and viruses are known to metabolize some fluoride compounds, but the ability varies among species. There is no evidence that fluorides are essential to microorganisms. As in higher organisms, excessive fluorides are toxic. The tolerance levels vary widely among organisms. Resting or reproductive stages such as spore forms are much more resistant to effects of excessive fluoride than are vegetative cells. Some organisms can adapt to gradual increases in environmental fluoride levels and increase their tolerance levels; others cannot.

Many experiments that showed detrimental effects to various microorganisms were conducted with fluoroorganic compounds. Whether the effects noted were due to the presence of fluoride alone or because of some biochemical effects of the entire compound is open to speculation.

Organisms may accumulate fluorides with no observable effects, but some, particularly lichens, may develop visible lesions that vary with species and amounts of fluoride accumulated. Lichens have thus been used as indicators of fluoride air pollution.

Toxic effects that have been reported include: developmental alterations and morphological changes, growth and reproduction inhibition, and (in applicable species) reduction in infectivity. Additional effects may include inhibition of respiration, carbohydrate catabolism, protein synthesis, and photosynthesis.

9.5 VEGETATION

Fluorides are in almost all vegetation in small amounts and may be essential to most vegetative growth. Usual concentrations in vegetation grown in normal soils, in unpolluted areas, range from about 2 to 20 ppm fluoride of dried plant material. In some industrial areas, the concentration in vegetation may be 10 to 20 times greater than normal. Fluorides are emitted in both gaseous and particulate forms from some industrial operations. Hydrogen fluoride and silicon tetrafluoride are the predominant gaseous forms and the forms most injurious to crops. Some vegetation seems to accumulate fluorides in amounts roughly proportional to the concentration in air. There is no such correlation between fluoride concentration in the soils and fluoride accumulation in vegetation grown on these soils.

One effect of fluoride in vegetation is the unseen buildup and concentration of fluoride in and on the vegetation. This affect may not be as well related to air quality as are the effects of foliar symptoms, growth, and physiology. The ingestion of vegetation containing high levels of fluoride may adversely affect animals even though the plants evidence no visible changes.

9.5.1 Sources of Fluorides to Vegetation

Fluorides in the soil are usually not in a chemical form that can be readily assimilated by vegetation. Soils near or above neutrality, those that are calcareous, and those high in clay retain fluoride in forms not generally available to plants.

The nature of the plant species and the availability of major nutrients in the soil also affect the amount of fluoride absorbed through roots. There is not a simple direct relationship between concentrations of fluoride in soil and in plant tissue because fluoride availability is strongly pH dependent.

Vegetation may become contaminated with fluorides when fluoride-containing soil is splashed onto the foliage by rain or sprinkled irrigation water. Also, windblown dust from surface soils high in fluorides may contaminate the vegetative parts that animals consume.

Usually waters contain less than 1 mg/liter fluoride although some geothermal and effluent waters may contain many times this amount. Most industrial effluent waters now have contamination limits imposed by state and federal laws. Limited quantities of these waterborne fluorides may reach plants through their roots, but considerable quantities may be absorbed from the water or adhere on the foliage when crops are sprinkler irrigated. Waters high in fluoride when applied to the soil leave their fluoride fixed on the surface soils, thus increasing the fluoride content. Dust or splash from these surface soils can add fluoride to the foliage of the crops. This is an indirect route of fluoride from the water to the plant.

Fluorides are released to the atmosphere in various ways: from volcanic activity and geothermal wells; from the manufacture of brick, fertilizers, ceramics, aluminum, and steel; from coal combustion; and in the form of gaseous hydrogen fluoride, silicon tetrafluoride, and fluoride particulates. In rural and urban areas, atmospheric fluoride concentrations are usually less than 0.05 mg/m^3. Localized atmospheric concentrations near industrial operations may be much higher. Most of the recognized damage to vegetation attributed to fluoride is caused by the absorption of gaseous fluorides (HF and SiF_4) through the leaves. Tissue-fluoride content increases with increased time of exposure and with increased atmospheric concentrations.

9.5.2. Fluoroorganic Compounds

More than two dozen exotic plant species are known to synthesize fluoroorganic compounds. Notable among these are *Dichapetalum cymosum* from Africa and *Acacia georgina* from Australia. Both of these plants can remove inorganic fluorides from the soil and convert them, in lethal amounts, to compounds such as fluoroacetate within the plant. Such compounds are extremely toxic to animals, but the plants are not adversely affected. It has been reported that, under certain conditions, fluoroacetate may also be synthesized by some cultivated crop plants such as soybeans and crested wheat grass; however, this observation is not well established. Fortunately, as far as is known, the accumulation of fluoroorganic compounds in these and other cultivated plants is only in trace quantities.

9.5.3 Symptoms and Susceptibility

The expression of inorganic fluoride-induced foliar lesions in vegetation varies considerably, depending upon the class of vegetation (narrow-leaved and broad-leaved), its relative susceptibility to fluoride, the concentration in the air, and duration of exposure. The symptoms produced in a given plant by a high fluoride concentration for a short duration may be different from those produced by a low fluoride concentration for a long duration.

Plants exhibit an extremely broad range of tolerances to fluoride, as well as to other air pollutants. Great differences in susceptibility are found, not only among different species, but also among varieties of the same species. The resistance of species such as camellia, cotton, and celery to fluoride may be contrasted to the extreme susceptibility of gladiolus, Chinese apricot, Italian prune, and developing needles of ponderosa pine. Differential responses may also be found among varieties of gladiolus, sweet corn, and sorghum, although the degree of difference is not as great. In gladiolus, dark-flowered varieties have been reported to be more resistant to fluoride than the lighter-flowered varieties. A similar situation has also been observed among certain conifers, such as ponderosa pine, that have phenotypic variants among the natural population showing differences in their responses to fluoride.

Specific and varietal differences in susceptibility have been tabulated by various researchers based on the following information: the amount of injury induced by a given dose of fluoride; the dosage of fluoride required to reach the threshold for foliar markings; and the amount of fluoride present in the leaves showing injury. These different means of estimating susceptibility account at least in part for the anomalous positions of several species in the tolerance ratings.

9.5.4 Effects on Vegetation

The effects of fluoride on vegetation depend on species and varietal differences in susceptibility, the stage of development of the vegetation, the concentration of fluoride in the air, the length of exposure, and climatic factors.

Excess fluoride may cause growth inhibition, tip and marginal necrosis of foliage (Figure 9.2), chlorosis, wilting, and eventual death of the plant. Fruit quality and yield can be impaired. Inhibition of seed germination may also occur. Fluoride causes these effects by altering photosynthesis, carbohydrate metabolism, respiratory and oxidative processes, RNA metabolism, and calcium nutrition. The mechanisms involved are complex and largely unresolved in vivo, but in vitro studies suggest that many effects result from fluoride inhibition of essential enzymatic reactions.

Excessive fluorides appear to exert mutagenic effects in some plants. Chromosomal abnormalities, such as breakage, bridging, and stickiness of mitotic cells, occur in a variety of plants after treatment with concentrations of gaseous hydrogen fluoride or aqueous sodium fluoride that are too low to induce immediate visible injury. The mechanisms by which fluorides induce chromosomal aberrations are not known.

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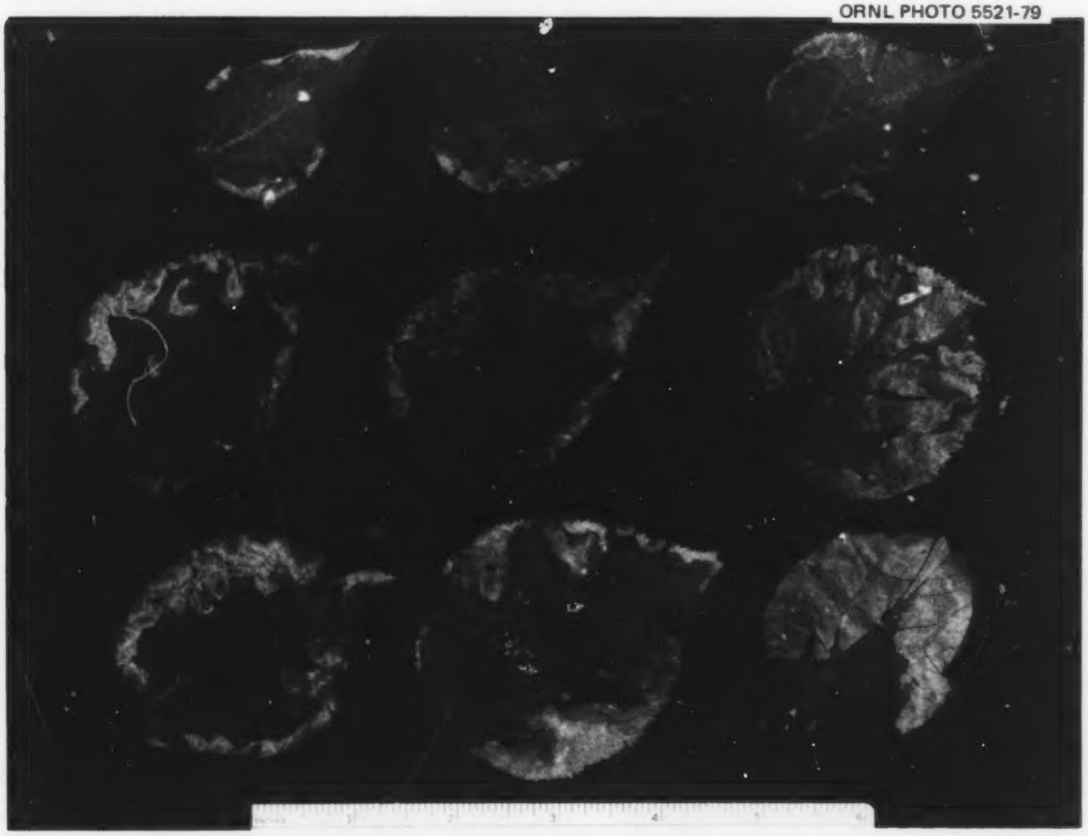

Figure 9.2. Apricot leaves showing various degrees of necrosis caused by excessive levels of atmospheric fluoride.

There are few data on possible synergistic effects on vegetation of fluoride combined with other toxicants. Limited experiments with HF and SO_2 indicate that the effects are additive in respect to reduction of linear growth and leaf area on orange and mandarin trees. Experiments also show that the inhibition of photosynthesis in alfalfa by NO_2 and HF is additive. Inhibition of photosynthesis, with combined SO_2 and HF, is somewhat more than additive.

When evaluating the effects of fluoride on a crop, consideration should be given to the intended use of the crop. For example, Douglas fir trees intended for lumber may exhibit needle tip burn but growth may not be inhibited, and there is no known economic loss. The same kind of trees grown as ornamentals, with the same symptoms of tip burn would be aesthetically unacceptable and hence damaged. Gladiolus with fluoride-induced markings on the leaves and flowers are unsalable, but if grown for corm production there may be no reduction in either yield or quality.

In addition to recognition of the characteristic fluoride-induced markings on crops in the field, there must also be recognition of similar symptoms induced by other agents. Marginal and tip necrosis typical of fluoride markings on leaves of many species may be similar to symptoms of water stress, low temperature, overfertilization, and toxicity from road salt. Fluoride-induced chlorotic patterns are mimicked by deficiencies of manganese, zinc, magnesium, calcium, boron, potassium, and iron; by virus infection, genetic disorders, mite or leafhopper-induced stippling; and herbicides. It is important to reinforce field observations with fluoride analyses of vegetative materials.

Sampling and analysis of vegetative materials are usually done for one or more of the following purposes: (1) to determine the amount and distribution of airborne fluorides, (2) to determine the levels of fluoride in and on vegetation to be utilized as animal feed, and (3) to determine the fluoride concentrations in vegetation that are harmful to the plants. Although theory may dictate the sampling scheme and the location of sampling sites, the biological or agronomic characteristics of the vegetation should determine the amounts and types of samples to be taken. Specific practices cannot be recommended for vegetation sampling and analysis unless the purpose of the sampling is identified. For any given purpose, the methods of sampling and analyzing should be standardized.

9.6 DOMESTIC AND WILD ANIMALS

Animals normally ingest variable low-level amounts of fluoride with no known adverse effects. It appears that trace amounts of fluoride are essential to or at least enhance certain body functions such as optimum tooth and bone development and mineralization. Growth and reproduction in some laboratory animal species are reported to be improved by fluoride ingestion at proper concentrations. Much of the published information, however, describes the adverse effects of excessive fluoride ingestion.

9.6.1 Sources of Fluorides to Animals

Both terrestrial and aquatic animals are exposed to fluorides in various forms and concentrations in their food, water, and air. Ingestion is the major mode of intake by most terrestrial mammals, birds, and lower animal forms. Aquatic forms acquire fluorides from their food and their environment. The most commonly encountered sources of excessive fluoride for higher animals are the following: (1) forages which usually constitute the major source of an animal's diet and that have been contaminated by fluoride-laden industrial effluents or by wind-blown or rain-splashed soil of high fluoride content; (2) water high in fluoride content because of natural (usually geothermal) or industrial sources; (3) feed supplements and mineral mixtures that have not been properly defluorinated; (4) forages grown in soils high in fluoride content; and (5) a combination of the various sources.

Many cases of fluoride toxicosis have resulted when airborne effluents, high in fluoride, have contaminated vegetation subsequently eaten by animals. Some cases of fluoride toxicosis have been induced by animals drinking waters with excessive fluorine from hot or warm springs, deep wells, geysers, or waters accidentally contaminated. Phosphorus supplements that have not been properly defluorinated have also caused toxicosis.

Soils usually contain relatively high amounts of fluoride without translocation of excessive fluoride into the vegetation. However, animals grazing such areas can ingest excessive fluoride from the soil, especially if the pastures or ranges are overgrazed and the animals are forced to eat the vegetation close to the ground or vegetation contaminated by wind or rain splash.

9.6.2 Fluoride Toxicosis

Fluorides in animals are to some extent cumulative, but only in mineralizing tissues. When ingested in excessive amounts fluorides induce characteristic lesions. The expression of severity of fluoride toxicosis may be altered or influenced by several factors including: (1) amount of fluoride ingested; (2) duration of fluoride ingestion period; (3) solubility (and thus relative toxicity) of ingested fluorides; (4) variations in fluoride ingestion levels (intermittent exposure); (5) species of animal; (6) age at time of ingestion; (7) level of nutrition; (8) exposure to other toxic agents (synergism or alleviation of effects); (9) general state of health of the animal; (10) stress factors; and (11) varied biologic response by different individuals within a group.

9.6.3 Signs and Lesions

Fluoride toxicosis may be either acute or chronic. Fortunately, acute fluoride toxicosis is relatively rare and most often results from accidental inclusion of toxic fluoride compounds such as sodium fluorosilicate or sodium fluoride in the diet of domestic animals. The rapidity with which signs appear and the exact nature of the signs depend mainly on the amount and type of fluoride ingested. Clinical signs may include restlessness, stiffness, anorexia, excess salivation, nausea, vomiting, incontinence of urine and feces, reduced milk production in lactating animals, convulsions, weakness, severe depression, and cardiac failure. Chemical analyses of blood and urine from affected animals reveal high fluoride content. Necropsy findings are usually limited to mucosal necrosis of the digestive tract.

The much more common chronic fluoride toxicosis in animals develops gradually and is usually insidious in nature. Time intervals of months or even years may pass between the beginning of excess fluoride ingestion and the manifestation of readily observable clinical signs. Some manifestations of fluoride toxicosis may be confused with other toxicoses or certain debilitating or degenerative diseases such as malnutrition or arthrosis.

9.6.3.1 Dental Fluorosis — The most distinctive and obvious primary lesions of chronic fluoride toxicosis appear in developing permanent teeth and bones. Developing teeth are extremely sensitive to excessive fluoride. Once the teeth have formed, mineralized, and erupted, fluoride ingestion will have little or no discernible effect on them. Thus, immature animals are most susceptible to dental fluorosis. Severely affected teeth are discolored (chalky white or creamy yellow to brownish black); they may also have hypoplasia, pitting, and loss of enamel with exposure of the dentine and are more susceptible to increased attrition.

Classification or evaluation of clinical dental fluorosis is usually recorded as numerical values (Figure 9.3). The most widely used classification system includes a range from 0 (normal) to 5 (severe fluoride effects). The incisor teeth are evaluated for enamel quality, defects,

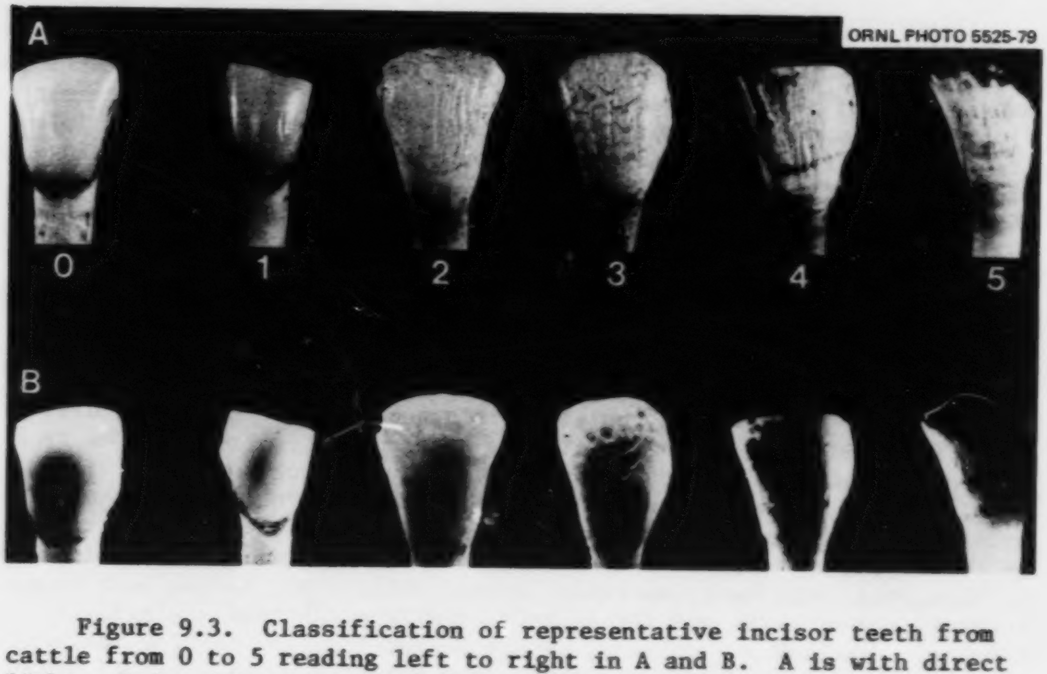

Figure 9.3. Classification of representative incisor teeth from cattle from 0 to 5 reading left to right in A and B. A is with direct light. B is with transmitted light. Source: Shupe et al., 1962.

and abrasion pattern (Figure 9.4). The more difficult to examine cheek (premolar and molar) teeth are classified 0 to 5 based on attrition patterns only.

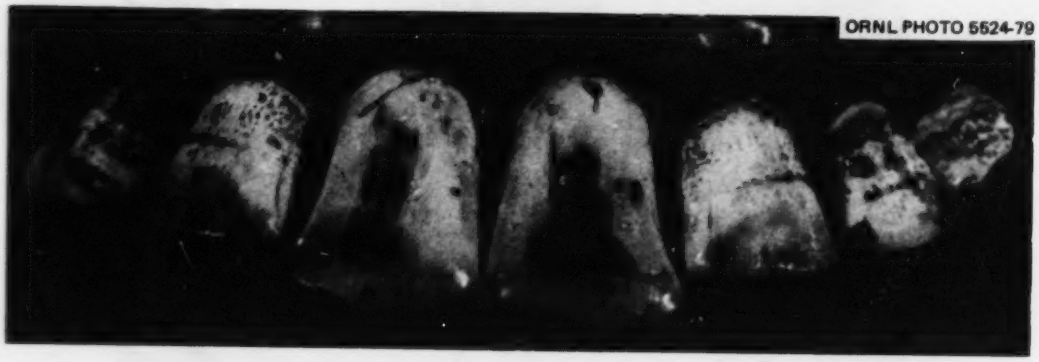

Figure 9.4. Permanent bovine incisor teeth. Note chalkiness and discoloration of enamel, hypoplasia, and excessive bilateral abrasion.

Dental lesions should be carefully examined and the incisor and cheek tooth lesions correlated for their bilateral nature and chronologically similar lesions relating to the animal's age at the time of formation and mineralization of the individual teeth.

9.6.3.2 Osteofluorosis — Fluoride toxicosis is also manifested by gross, hyperostotic chalky white bone lesions if fluorides are ingested at high enough levels for a prolonged period (Figures 9.5 and 9.6). These lesions can be induced at any time during an animal's life. Severe osteofluorosis has been reported in animals with normal permanent teeth, thus accenting the significance of the age-effect factor in relation to induced lesions.

Fluoride-induced bone lesions are generally bilateral and somewhat symmetrical. The severity of the bone lesions is related to the structure and function of the bones involved and to the amount of stress and strain placed on specific areas in the bones. Affected bones usually are chalky white and have a roughened irregular periosteal surface. They may also be larger in diameter and heavier than normal. The major bone changes are located on the periosteal surface. In some advanced cases, endosteal proliferation may occur with encroachment of bone and the marrow cavity of some bones. If the lesions are severe and of long enough duration, some secondary periarticular and intraarticular changes may occur. The severity and exact nature of the fluoride-induced bone changes varies, and one or more of the following conditions may occur: osteosclerosis, osteoporosis, hyperostosis, osteophytosis, or osteomalacia. The above listed changes such as increased density, porosity, hyperostosis, or osteophytosis may be demonstrated radiographically as well as grossly.

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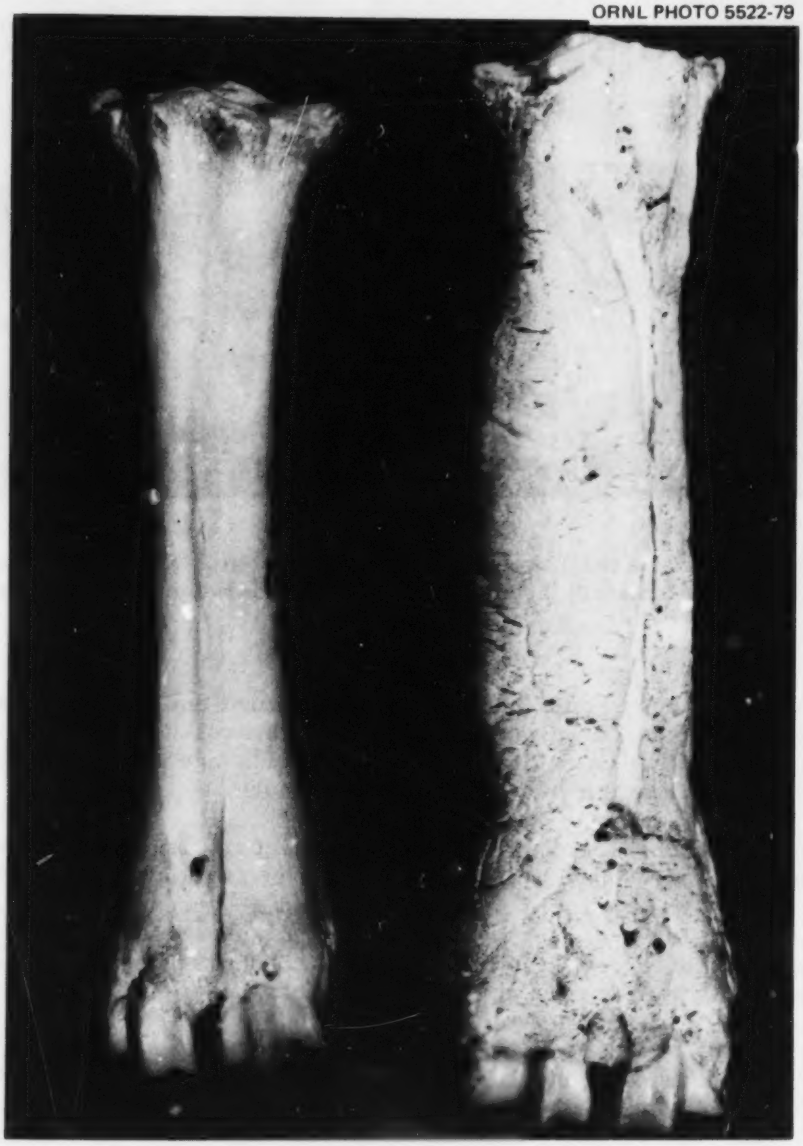

Figure 9.5. Metatarsal bones from two cows of the same breed, size, and age: left — normal bone; right — severe osteofluorosis.

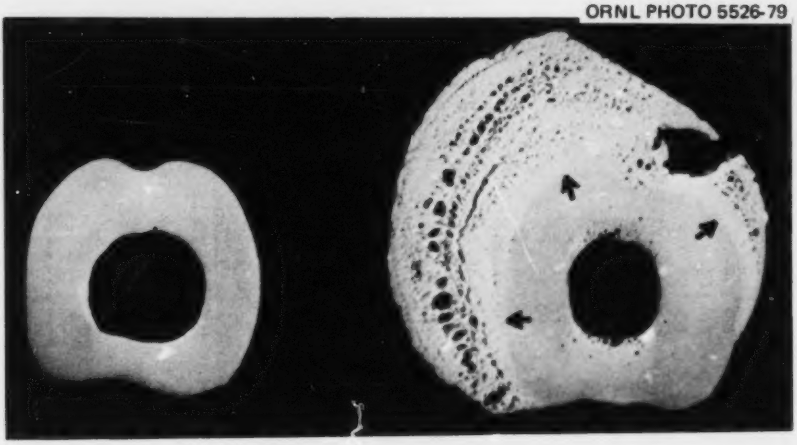

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Figure 9.6. Cross sections of the two metatarsal bones shown in Figure 9.5. Bone from points of arrows outward is fluoride-induced abnormal bone.

Osteones formed under the influence of excessive fluorides frequently vary in size and shape with osteocytes often clumped or irregularly located near the periphery of the osteone rather than evenly distributed throughout the osteone. Abnormal canaliculi are associated with the clumped abnormal osteocytes. Porous areas with excessive bone resorption or accelerated remodeling occur with failure of bone growth and remodeling to keep a proper balance between bone destruction and formation.

The precise pathogenesis of osteofluorosis is not fully understood. There appear to be three phases associated with osteofluorosis: (1) elevated fluoride levels in the bone without detectable structural changes; (2) microscopic and radiographic bone changes without known alteration of function; and (3) sequential and progressive structural bone changes that result in abnormal bone structure and function with resultant alteration of mechanical properties.

9.6.3.3 Other Signs — Animals with moderate to severe osteofluorosis sometimes exhibit an intermittent, nonspecific, atypical lameness or stiffness that may be associated with calcification of periarticular structures and tendon insertions. This lameness or stiffness is often transitory and intermittent in nature and limits grazing or feeding time, thereby impairing animal performance. The type of lameness or stiffness seen is not diagnostically specific for fluoride toxicosis. Other general nonspecific signs sometimes associated with chronic fluoride toxicosis include unthriftiness, thickened dry unpliable skin, and signs of poor performance.

9.6.3.4 Tissue Analyses — Locations from which samples are taken should be standardized and consistent. Fluoride content of bones normally increases with age (Table 9.1) even if the animal is on a lifelong low fluoride intake level. Usually 96% or more of the fluoride in an animal's body is located in the bones and teeth.

TABLE 9.1. GUIDE TO DIAGNOSIS AND EVALUATION OF FLUORIDE EFFECTS IN DAIRY CATTLE[a]

Parameter	Age (years)	Normal conditions	No adverse effects	Chronic fluoride toxicosis			Acute fluoride toxicosis[b]
				Threshold	Moderate	Severe	
Fluoride in moisture-free diet, ppm	2	Up to 15	15-30	30-40	40-60	60-109	Over 250
	4	Up to 15	15-30	30-40	40-60	60-109	Over 250
	6	Up to 15	15-30	30-40	40-60	60-109	Over 250
Degree of fluoride effects on incisors[c]	2	0-1	0-2	2-3	3-4	4-5	
	4	0-1	0-2	2-3	3-4	4-5	
	6	0-1	0-2	2-3	3-4	4-5	
Degree of wear on molars[c]	2	0-1	0-1	0-1	0-1	0-3	
	4	0-1	0-1	0-1	1-2	1-4	
	6	0-1	0-1	1-2	1-3	1-5	
Fluoride in bone, ppm on a dry, fat-free basis	2	401-714	714-1605	1605-2130	2130-3027	3027-4206	
	4	706-1138	1138-2379	2379-3138	3138-4504	4504-6620	
	6	653-1221	1221-2794	2794-3788	3788-5622	5622-8676	
Fluoride in urine, ppm	2	2.27-3.78	3.78-8.04	8.04-10.54	10.54-14.71	14.71-19.86	
	4	3.54-5.3	5.3-10.32	10.32-13.31	13.31-18.49	18.49-25.63	
	6	3.51-6.03	6.03-11.29	11.29-14.78	14.78-20.96	20.96-30.09	
Fluoride in milk, ppm	2	Up to 0.12	Up to 0.12	0.12-0.15	0.15-0.25	0.25 and above	
	4	Up to 0.12	Up to 0.12	0.12-0.15	0.15-0.25	0.25 and above	
	6	Up to 0.12	Up to 0.12	0.12-0.15	0.15-0.25	0.25 and above	
Fluoride in blood, ppm	2	Up to 0.30	Up to 0.30	0.30-0.40	0.40-0.50	0.50 and above	
	4	Up to 0.30	Up to 0.30	0.30-0.40	0.40-0.50	0.50 and above	
	6	Up to 0.30	Up to 0.30	0.30-0.40	0.40-0.50	0.50 and above	
Fluoride in soft tissue, ppm	2	Up to 1.20	Up to 1.20	Up to 1.20	Up to 1.20	Up to 1.20	
	4	Up to 1.20	Up to 1.20	Up to 1.20	Up to 1.20	Up to 1.20	
	6	Up to 1.20	Up to 1.20	Up to 1.20	Up to 1.20	Up to 1.20	
Periosteal hyperostosis[c]	2	0	0-1	0-1	0-2	0-3	
	4	0	0-1	0-1	0-3	0-4	
	6	0	0-1	0-2	0-4	0-5	
Secondary changes[d]	All	Absent	Absent	Occasionally noticed	Present	Present	

[a] Data based on controlled experiments ranging from 1.5 to 7.5 years and have been correlated with 855 fluoride-affected field cases that were necropsied and evaluated.

[b] Characterized by: excitement, high-fluorine content of blood and urine, stiffness, anorexia, reduced milk production, excessive salivation, nausea, vomiting, incontinence of urine and feces, clonic convulsions, necrosis of mucosa of digestive tract, weakness, severe depression in some cases, and cardiac failure.

[c] 0 — normal, 1 — questionable, 2 — slight, 3 — moderate, 4 — marked, and 5 — severe.

[d] Stiffness and lameness, loss of body weight, reduced feed intake, rough hair coat, unpliable skin, and reduced milk production.

Fluorides do not normally accumulate in visceral organs or soft tissues in sufficient quantities to provide reliable, accurate, and consistent diagnostic information. Neither have fluorides been shown to induce consistent demonstrable changes in the visceral organs or soft tissues. Therefore, meat from fluoride-affected animals is safe for human consumption and will usually contribute only negligible amounts to the total human intake. Edible marine animals, particularly crustaceans, growing in an environment that is usually quite high in fluorides will accumulate more fluorides in both edible tissues and exoskeletons than will many terrestrial dwellers. Even the larger amounts of fluoride that these edible tissues will contribute to some human diets is not great enough to cause any concern unless there are also other significant fluoride sources. Milk from dairy animals consuming high fluoride levels will have a fluoride content only slightly above normal, and the milk is safe for human consumption (Table 9.1). The mammary gland is not a major excretor of fluorides. Some fluoride does cross the placental barrier to the fetus but usually in only small amounts. No experimental evidence has indicated any discernible fluoride-induced damage to fetuses of mammals on a relatively high fluoride intake. Fluoride buildup in the food chain of higher animals has not been detected.

9.6.3.5 Urine — For domestic animals, urine samples, if properly collected and analyzed for fluoride, can provide supportive data assisting in the diagnosis and evaluation of chronic fluoride toxicosis. Individual random samples may be too variable to provide reliable information.

In mammals, urine serves as one of the major means of fluoride elimination from the body. On a low-level fluoride diet the urinary fluoride content from animals rises slightly as animals age and the fluoride content of bone increases. Table 9.1 depicts the relationship of age and level of fluoride ingestion to fluoride content in bone, urine, milk, blood, soft tissues, and fluoride-induced lesions. Urinary levels of fluoride may remain elevated for some time after animals are changed from a high to a low fluoride intake. Under most circumstances, urine values for cattle of less than 6 ppm fluoride are considered normal.

9.6.3.6 Necropsy Findings — If clinical examinations and chemical analyses indicate the need for more specific information, necropsies should be performed on selected representative animals. For economic reasons, some domestic animals may be sacrificed at a commercial abattoir and after recommended tissues are taken for detailed study and evaluation, the carcasses could be safely used for meat.

9.6.4 Diagnosis

Results of clinical examinations should be compared with necropsy findings. Chemical analyses, especially of bones, reflect past exposure to fluoride. All feed sources, including mineral supplements, should be analyzed for fluoride content. Likewise all drinking water supplies should be tested so that the cause(s) of fluoride toxicosis may be elucidated. Sampling and analytical techniques should be standardized and recorded to insure reliable and reproducible data.

In regard to forage crop sampling, extensive efforts should be made to accurately sample the forage the involved animals have actually been ingesting.

There is considerable experimental information on the tolerance of livestock to fluoride in feed when animals are housed and managed under ideal experimental conditions. There is a paucity of information on tolerances of animals under field conditions when they may not be ideally fed and properly sheltered or managed. Information on tolerances of some animals to waterborne fluoride is also limited. Response of animals to waterborne fluoride varies as ambient air temperature influences the amount of water consumed. Figure 9.7 illustrates the relation of ambient air temperature and water intake by beef cattle as related to dietary dry matter intake.

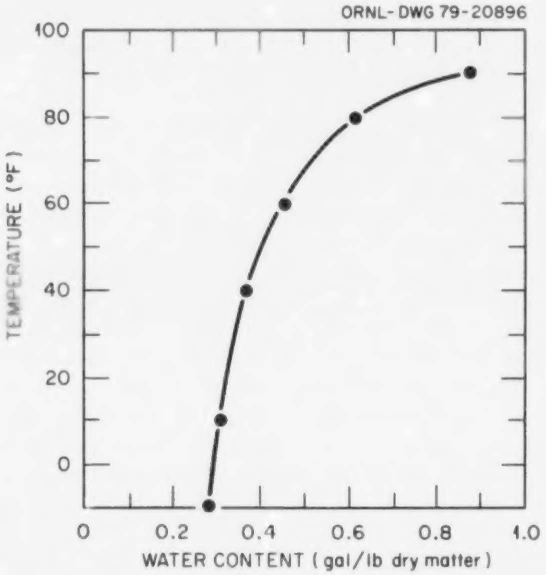

Figure 9.7. Water intake by beef cattle at different ambient air temperatures.

Overall husbandry or management practices may also affect the expression of fluoride toxicosis. A complete clinical history of each animal should be obtained. Such things as seasonal changes in rangeland grazing patterns or variable feed sources may induce intermittent excessive fluoride exposure in animals. Other trace element toxicoses or deficiencies may, in some ways, alter or mimic some aspects of chronic fluoride toxicosis. Some debilitating diseases such as degenerative arthrosis may also mimic certain aspects of fluoride toxicosis.

For wildlife the diagnosis, treatment, or prevention of fluoride toxicosis is much more difficult and in many cases it is impossible to follow and utilize the procedures used for domestic animals.

9.6.5 Treatment

There is no known successful treatment for chronic fluoride toxicosis. Supportive and symptomatic treatment may be given. Complete recovery from moderate to severe lesions induced by excessive fluoride ingestion does not occur. If dental lesions are present, they are irreversible. In fact, if lesions are severe enough to hasten dental abrasion, the accelerated wear will continue, especially if rough abrasive feeds are utilized.

Osteofluorotic lesions may be prevented by reducing to normal the total fluoride intake. Animals with extensive bone lesions do not live long enough to have the bone remodeled to normal. After animals stop ingesting excessive fluoride levels, normal bone is laid down over previously induced osteofluorotic bone.

Animals adversely affected by fluoride toxicosis may be aided by providing good nutrition and by avoiding all possible stresses. The diet should be nutritionally well balanced, of good quality, and in sufficient amounts to promote desirable performance and growth.

If economic or logistic feasibilities dictate the animals may not be removed from toxic sources of fluorides, some preventive relief may be obtained by feeding alleviating compounds. Beneficial effects have been observed by feeding adequate mineral supplements and adding calcium carbonate, magnesium, or aluminum compounds to the diet. Inclusion of some aluminum (most often aluminum sulfate) or calcium (calcium carbonate) compounds in the diet has lessened the effects of a given amount of fluoride. A nutritional balance of other minerals, particularly phosphorus, should be maintained to obtain maximum benefits from aluminum and calcium additions.

9.6.6 Species Tolerances

Tolerance levels for various livestock species have been determined. Where applicable, different levels for long-term production/reproduction animals as compared to short-term fattening/slaughter animals have been calculated and can be found in Table 9.2. The values given are for sodium fluoride or for compounds of similar solubility (toxicity) in the total dietary intake. Less soluble fluoride compounds, such as calcium fluoride, are less toxic on a given parts-per-million fluoride basis.

Fluorides in water, particularly those occurring naturally, are often dissolved inorganic forms that are biologically available. Fluorides in water are often relatively more toxic than equivalent amounts in the dietary dry matter. Many fluoroorganic compounds have the fluorine ionically bound and biologically unavailable. These variations in biologic availability and relative toxicity of fluoride forms and sources present must also be taken into consideration when evaluating fluoride toxicosis.

TABLE 9.2. FLUORIDE TOLERANCE LEVELS IN FEED AND WATER FOR DOMESTIC ANIMALS BASED ON CLINICAL SIGNS AND LESIONS[a]

Species	Feed[b] (mg/kg)	Water[c] (mg/liter)
Cattle		
Dairy and beef heifers	30	2.5-4.0
Dairy, mature	40	3-6
Beef, mature	50	4-8
Finishing	100	12-15
Sheep, breeding	60	5-8
Lambs, feeder	150	12-15
Horses	60	4-8
Swine, growing	70	5-8
Turkeys, growing	100	10-12
Chickens, growing	150	10-13
Dogs, growing	50	3-8

[a] Values should be reduced proportionally when both water and feed contain appreciable amounts of fluorides.

[b] A suggested guide when fluoride in the feed is essentially the sole source of fluoride, tolerances based on sodium fluoride or other fluorides of similar toxicity.

[c] The average ambient air temperatures and the physical and biological activity of the animals influence the amount of water consumed and hence the wide range of tolerance levels suggested. For active animals in a warm climate, the lower values should be used as critical level indicators.

Fluoride tolerance levels for other mammalian species and for lower animal species are generally less well documented or nonexistent and often more variable than those reported here. The effects of waterborne fluorides on fish have been studied somewhat. Hatching time of fish eggs is extended in high fluoride waters. Effects of fluorides on growing fish varied with species, size at exposure time, water temperature, and calcium and chloride levels in the water. The above factors, plus water pH and the presence of other contaminants, influence the tolerance and overall effects of fluorides on aquatic animals. Some reports indicate that aquatic animals can usually tolerate fluoride levels up to 1.5 ppm in the water. The effects of higher fluoride levels are variable. Low levels appear to stimulate growth rates, but higher levels slow egg hatching and growth and are also otherwise toxic in some species. Edible tissues in aquatic animals, particularly crustaceans, will accumulate much greater fluoride levels than will terrestrial mammals — up to 50 ppm have been reported in some studies. Reversal of fluoride storage or accumulation in aquatic animals occurs much more rapidly than in terrestrial dwellers. Most reports cite return to normal levels in approximately the same length of time as it took the high levels to accumulate after the animals' water supply returned to a low fluoride level. Tolerance levels for some animal species are difficult to establish because of the many variables involved and the limited research conducted.

Effects of fluorides on insect species are highly variable. Levels of sodium fluoride (0.01%) mixed in flour have been shown to stimulate egg production in one type of beetle (*Tribolium* sp.) often found in cereal products, while 0.1% of the same compounds will inhibit egg production. One organic fluoride compound (perfluorobutene-2) mixed with air in a 1:9 ratio and used as an air source for fruit flies (*Drosophila* sp.) increased mutations in the offspring. The close ionic bonding of such compounds, however, limits the likelihood of the fluorine alone being the chief toxic principle. Reports of fluoride toxicosis in honeybees have been published. These incidents usually involved errant pesticide applications with an acute response and high mortality within two to three days of exposure. Like many other reports, the amount of fluoride involved in bee fluoride toxicosis is variable.

9.6.7 Prevention of Fluoride Toxicosis

Caution must be exercised by animal owners, animal-feed processors, and industries to prevent the occurrence of fluoride toxicosis. All sources of feed and water should not contain fluoride in excess of normal recommended levels. It is very important to avoid overgrazing by livestock so that chances of ingesting high-fluoride soils are lessened.

Fluorine in any mineral or mineral mixture that is to be used directly for the feeding of domestic animals should not exceed 0.20% for dairy and breeding cattle, 0.30% for slaughter cattle, 0.30% for sheep, 0.30% for horses, 0.35% for lambs, 0.45% for swine, and 0.60% for poultry. The total intake is of major importance and when total fluoride intake is calculated, it should include both feed and water intake as recommended in Table 9.2.

One must also be aware of potential industrial pollution from new industries in an area or from long-established industries that change processes or methods of operation. Another area of recent concern is the rapid expansion of sprinkler irrigation with geothermal waters. Some waters are safe for use in flood irrigation, but unpublished data indicate a high fluoride content in forage sprinkled with water containing high levels of fluoride (5 to 10 mg/liter).

Owners of domestic animals and wildlife managers should carefully observe all phases of animal health and be aware of problems that may occur with infectious agents, nutritional imbalances, and now, more than ever, with toxicities of all kinds including fluoride toxicosis.

Measures should be taken to exclude domestic animals and wildlife from as many sources of excessive fluoride as practical. Prevention and control of fluoride toxicosis in animals can be achieved when the complexity of the disease is realized, properly diagnosed, and the source(s) of excessive fluorides is eliminated.

9.7 HUMANS

Proper amounts of ingested fluoride are beneficial to man. Dental benefits are maximized when water containing approximately 1 ppm (1 mg/

liter) fluoride is consumed during the years of tooth formation and continued throughout life. This conclusion is based on epidemiological studies conducted in areas where fluoride occurs naturally in the water and in other areas where it has been added at the recommended level to public culinary water supplies. Some fluoridation programs have been in operation since 1945 and have been successful in reducing and/or, in some subjects, preventing dental caries.

Only when massive (e.g., 20 to 80 mg or more per day) dosages of fluoride are taken in over long periods (10 to 20 years) are adverse effects encountered. No adverse effects have been reported when water containing 1 ppm of fluoride has been drunk during periods of 10 to 20 years. Fluoride-induced mottling of tooth enamel having only cosmetic effects has been reported only when fluoride concentration in the water exceeded 1.4 to 1.6 ppm, and it was minimal at such levels.

Research has established that people consuming water containing 1 ppm of fluoride experience no adverse effects on their kidneys, thyroid glands, reproductive functions, growth, development, blood, urine, or hearing. No cases of allergic reactions have been positively linked with consumption of water fluoridated to the 1 ppm level.

Research has also provided evidence that suitable amounts of fluoride may be helpful in preventing or alleviating bone diseases such as osteoporosis.

Man is subject to similar tooth and bone effects from excessive fluoride ingestion as are other higher animals. However, detrimental effects are usually minimized by the nature of man's diet and habits and by dilution of fluoride sources because food and water are obtained from diversified sources.

Fluoride-containing compounds that are involved in human chronic fluoride toxicosis are almost exclusively inorganic forms. The fluoride is bound in compounds of varying solubility. Commonly encountered forms include calcium fluoride, sodium aluminum fluoride, and sodium fluoride. The adverse biological effects of a given compound are proportional to the solubility of the compound.

Fluoroorganic compounds do not mimic the physiological effects of inorganic fluorides. In most of the organic compounds the fluoride is tightly bound. Compounds that do not readily yield free fluoride ions in the body fluids have little or no toxicity that is due to their fluoride content alone.

Most wells and community water supplies naturally range from 0.01 or less to 10 (or rarely more) ppm fluoride. In some parts of Africa, brackish pothole waters must be used as culinary water supplies during periods of extreme drouth, thus increasing fluoride intake to a serious level.

The amount of fluoride irgested with water is dependent on the fluoride content of the water and the amount of water consumed. There is a great variation in both of these factors. Age, physical activity, and ambient air temperatures all affect water consumption rates.

Most human foods are minor sources of fluorine, even when grown in high fluorine soils. Many factors can influence the fluoride content and analytical results. Table 9.3 lists the fluoride content of some foods.

TABLE 9.3. FLUORIDE CONTENT OF VARIOUS FOODS

Food	Content (ppm)
Meat	0.2-2.0
Fish (fresh and canned)	0.1-30.0
Hen eggs	0.5-1.5
Cow milk	0.05-0.25
Tea (dried leaves)	3.0-180
Citrus fruits	0.07-0.36
Noncitrus fruits	0.06-0.22
Cereals and cereal products (most)	0.1 or less-0.3
Vegetables and tubers	0.08-0.8

Many foods contain less than 0.3 ppm fluoride on a fresh-weight basis, the exceptions being certain seafoods and tea. Most fluoride occurring in food is in chemical forms that can be assimilated by the body. An average American diet provides approximately 0.2 to 0.5 mg of fluoride daily.

Cow milk and meat (skeletal muscle) normally contain very little fluorine. High fluoride intake by the cow does not appreciably increase the fluoride content of the cow's milk or meat (Table 9.1).

In addition to the fluorides ingested in foods, minute amounts may also be ingested as fluoride supplement tablets, fluoride-vitamin and/or mineral supplements, fluoride-containing dentifrices or mouthwashes, or in fluoride-containing drugs. In the drugs, the fluoride is often contained in organic forms and metabolically unavailable.

Estimates of fluoride ingestion with diets range from about 0.2 to 2.7 mg per day worldwide with little variation shown from country to country. Some studies on U.S. per capita ingestion range from 0.2 to 0.8 mg of fluoride per day.

Uncontaminated air normally contains only minute quantities of fluoride that are measured in parts per billion (ppb). Results of fluoride analyses of air samples collected in various U.S. cities during the 1950s ranged from 0.04 to 1.8 ppb fluoride.

Hydrogen fluoride is the most widely encountered gaseous fluoride compound in the environment. It is widely used in certain industrial processes. When exposure is to high levels and for long periods of time, hydrogen fluoride may cause eye, skin, and respiratory system irritation.

Winds blowing over soils or rocks containing fluoride may move excessive amounts into the air, onto edible vegetation, or into culinary water supplies — and consequently into the food chain. Many industrial processes may also liberate fluoride-laden dusts or particulates into the atmosphere. The biologic effects of the fluoride intake from dust or particulate matter are much more closely related to ingestion of similar quantities of sodium fluoride than to inhalation of comparable amounts of gaseous hydrogen fluoride compounds.

Because elemental fluorine occurs only rarely, if ever, in nature, human exposure occurs only in some industrial manufacturing processes or in laboratories.

Aerosol propellants containing fluoroorganic compounds do not constitute a health hazard solely because of their fluoride content. They are a potential problem because of their possible reaction with the protective ozone layer in the upper atmosphere.

From a health standpoint, except for rare industrial exposures, the most significant exposures of man to atmospheric fluorides come not from inhalation but ingestion of dust or particulate-contaminated food and/or water. An average person breathing air containing 10 ppb fluoride, even with 100% retention, would only have an intake of 0.16 mg fluoride per day.

Ingested soluble fluoride compounds are rapidly absorbed in the gastrointestinal tract, primarily in the stomach. A high percentage of the fluoride in an average diet is absorbed. Less soluble fluoride compounds are absorbed to a lesser extent, with residual amounts being excreted in the feces. Diets rich in calcium, magnesium, or aluminum compounds, or low in fat, reduce absorption of fluorides. Soluble fluorides as gases or vapors, or as fine particle-sized dust can be readily absorbed from the respiratory tract. The absorption of hydrogen fluoride through the skin has also been reported.

Absorbed fluorides are rapidly distributed throughout the body via extracellular fluids. The level of fluoride in these circulating fluids is stabilized either by excretion or by storage of fluoride in bones and mineralizing tissue. Unless the body is absolutely overwhelmed by an acute dosage, stabilization occurs rather rapidly.

The principal route of fluoride excretion is via urine although some is excreted in sweat, especially from those who sweat profusely. After absorption, quantities of fluoride appear rapidly in the urine. The amount excreted is governed by many factors. Urinary fluoride concentrations can vary from hour to hour, even in the same individual. Therefore, single urine analyses may not accurately reflect a person's fluoride intake status. When fluoride intake is at a relatively constant level, human urinary fluoride concentrations are usually nearly equivalent to the drinking water concentrations.

The main portion of fluoride administered by any route, on entering the extracellular fluids, is sequestered by the skeleton and/or developing teeth and mineralizing tissue. Fluorine is the most exclusively bone-seeking element known. Fluorine incorporated into bone increases the size of the apatite crystals, or building blocks of bone, and decreases their solubility. The bones of persons on long-term low-fluoride intake levels, will gradually increase in fluoride content. Such low-level accumulations occur with no demonstrable changes in either bone structure or function. Skeletal tissues have a very high degree of physiological tolerance to the slow accumulation of low levels of fluorides. In experimental animals on fluoride-ingestion trials, the rate of fluoride uptake by bone decreases with time as bone activity and growth rate slow with maturation and/or aging. The bone crystal sites available for fluoride incorporation may also be reduced following near saturation with fluoride.

Fluoride deposition in the skeleton of man is not an irreversible process. If a person changes from an elevated to a low fluoride intake level, fluorides will be removed from the bone and excreted in the urine as normal bone remodeling proceeds.

In moderate amounts, fluorides tend to stimulate bone cells to produce a bone matrix that is better mineralized. This harder bone counteracts one of the most debilitating diseases of the elderly - demineralization of the skeleton or osteoporosis. It has been noted that osteoporosis in the elderly is much more rare in populations consuming adequately fluoridated water (as high as 8 ppm fluoride) than in the general population. Elevated fluoride-intake levels may disturb normal bone growth, development, and remodeling with resultant osteofluorosis. Poor nutrition increases the effects of a given fluoride intake level. Ligaments may calcify, and there may be extra bone development. This fluoride-induced bone may develop around joints and restrict movement, or develop as spur-like projections or roughened surfaces on long bones. Gross bone lesions only appear in humans who have had extreme chronic exposure to fluorides or (much more commonly) where effects of high fluoride intake have been modified or exaggerated by poor diet or malnutrition. Many residents of India and China exhibit greater effects of fluoride ingestion than their actual intakes predict that there should be, based on experimental studies of uncomplicated chronic fluoride toxicosis.

Fluoride ingestion levels have a lifelong effect on the skeleton. Bones are dynamic in that they constantly undergo growth, adaptive changes, repair, and remodeling. A proper, but constant fluoride intake, coupled with good nutrition, promotes a healthier skeletal system.

Developing teeth, particularly permanent teeth, are extremely sensitive to fluorides. Epidemiological and experimental studies have verified the association of fluorides, particularly waterborne fluorides at a concentration of approximately 1 ppm fluoride, with production of enamel that has significant resistance to decay. Significantly higher levels of fluoride have a converse effect. The enamel-forming cells (ameloblasts) are stimulated to form a poor enamel matrix with subsequent poor mineralization. How much ameliogenesis is affected depends on fluoride ingestion levels and other factors such as nutrition. Slight overexposure to fluorides results only in slightly hypoplastic enamel which, while not aesthetically pleasing, is actually harder and more resistant to wear and decay than normal enamel. Higher fluoride intake levels at the time of tooth development cause hypoplastic and discolored enamel which may also be softer than normal (Figure 9.8).

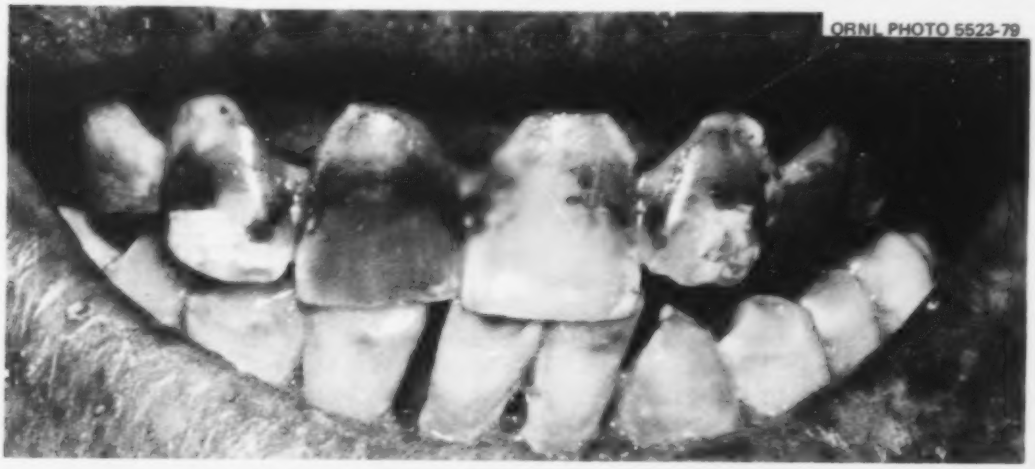

Figure 9.8. Permanent human teeth of a 55-year-old man. Note extensive discoloration and hypoplastic chalky white areas caused by the ingestion of water with high fluoride content during the period of tooth formation.

Once teeth have formed and erupted into the oral cavity, effects of excessive fluoride are minimal. After the ameloblasts have completed their formative work, they are incapable of reconstructive repair work, and continued fluoride exposure cannot lead to further enamel defects.

Fluorides do not accumulate in human soft tissues or organs in any appreciable amounts. Soft tissues are markedly unresponsive to an increased fluoride intake in terms of showing an elevation of fluoride content or alteration of function.

Dental benefits from the ingestion of optimally fluoridated water have been consistently reported through evaluatory surveys from 27 states in the United States and 9 other countries. More than 100 of these studies cover surveys ranging from 10 to 22 years. The results consistently show a marked reduction in the number of decayed, missing, and filled teeth; a reduction in the size of cavities which occur; and a definite increase in the number of caries-free children in those individuals receiving near the optimum fluoride level during the period of tooth formation.

The recommended optimum concentration of fluoride in water (0.7 to 1.2 ppm according to locality) has shown no impairment of, or adverse effect on, the general health status of individuals. No systemic abnormalities or abnormal laboratory findings have been noted that were associated with fluoride ingestion at the recommended level. Only when relatively large amounts of fluoride (8 to 20 mg or more per day) are ingested over periods of many years are any generalized adverse effects encountered.

9.8 RESEARCH NEEDS

Although extensive research has been conducted and voluminous literature compiled regarding the effects of fluorine and its compounds on biological systems, some aspects still need clarification and additional research.

9.8.1 Properties and Environmental Occurrences

There is a need for a better characterization of the amounts and the chemical and physical nature of airborne fluorides in suburban, urban, and especially the industrial atmospheres.

Research is needed to facilitate modeling the dispersion and fallout of airborne fluorides from industrial sources. Such information would be useful in predicting the accumulation of fluorides in vegetation, crops, and pastureland in the vicinity of industrial sources, as well as indicating human populations that might be exposed.

Currently employed methods for sampling and measurement of airborne and particulate fluorides alone or in combinations still leave much to be desired. Better standardization of methods and definition of sampling procedures would allow results to be more easily compared, extrapolated, and interpreted as to the total effect on the environment.

9.8.2 Environmental Interactions

Almost no information exists that describes possible interactions between gaseous and particulate fluorides or between fluoride and other pollutants. Such data would be valuable in predicting the degree and type of possible injury in various biological systems.

Some chemical combinations in biological materials interfere with analyses for fluorine. Standardized methods of dealing with these interfering factors need to be developed so that analytical results will be more consistent among laboratories. Ways to more effectively extrapolate analytical values (particularly from air sampling) to potential biological effects should be developed.

9.8.3 Microorganisms

There is a paucity of basic understanding of effects of fluorides on microorganisms. There is a wide array of microbial responses to fluoride exposure, but the effects of various governing factors under varied conditions and on different organisms need additional research.

It is known that: (1) certain fluoride levels will inhibit some enzymes; (2) certain bacteria can concentrate fluorides; (3) fluorides cause some microbial metabolic alterations; (4) some bacteria develop a resistance to fluoride effects; (5) there is potential for development of a whole array of fluoride-containing antimicrobial agents with unique powers of selective specificity; (6) some persistent fluorocarbons can be chemically split and decomposed by some bacteria; and (7) many other phenomena are known but the specific mode of action in these and many other instances is not fully understood.

Though the role of fluoride incorporated into developing teeth is known, the mechanism by which topical application of fluoride to teeth acts as a cariostatic agent is not known. Similarly, interactions of fluorides and other ions on the development of dental caries are not completely understood. Apparently fluorides alter the metabolism of oral microbes, but the mechanism is not understood.

Answering some of the basic questions of fluoride effects on microorganisms may lead to better understanding of fluoride effects on other life forms.

9.8.4 Vegetation

Additional understanding of the structural and physiological characteristics of vegetation sensitivity to or tolerance for fluorides is needed. Further studies could reveal the location of fluoride accumulations in the cells and its relation to mineral nutrition and other physiological reactions in vegetation.

Additional research is required in order to determine how various forms of fluorides influence the different species and varieties of plants grown under differing environmental conditions such as intermittent exposure. More information is needed regarding the amount of fluoride uptake by various plants, as compared to the concentrations of fluorides in the soils. The information gained would make possible extrapolation of more data from controlled experiments to practical field conditions. There is presently a paucity of data related to the beneficial and harmful effects of fluorides on the yield and quality of crops growing under field conditions.

With increasing demands for energy there has been an increased release of geothermal waters containing high levels of fluoride and utilized for crop production. In order to best manage the use of these and other effluent waters, research on fluoride accumulation, translocation, and injury to crops is needed. Of particular concern is the effect from use of high-fluoride waters for sprinkler irrigated crops. Minimizing the fluoride content of forage crops irrigated with such waters is of concern to the livestock industry.

9.8.5 Domestic and Wild Animals

Much is now known about various aspects of fluoride toxicosis in some animals, but our knowledge is far from complete.

Reports of fluoride effects are most often based on studies of confined experimental animals. Because of the many variables and factors involved in fluoride toxicosis, extrapolation of such experimental data to practical field situations is often difficult. Fluoride levels that can be tolerated under different environmental conditions need to be better defined.

There is a marked variation in tolerance levels reported in the literature for many lower animal forms, especially aquatic species. Much additional research is needed, under standardized conditions, to decrease the reported tolerance ranges.

The effects of interactions of fluorides with other possible toxicants warrant substantial study in all species. These elements may occur together naturally, yet little is known of their combined effects.

The effects of stress, whether from the environment, poor management practices, or other factors such as nutritional inadequacies, need more research.

[illegible] it is recognized that some substances, particularly certain aluminum and calcium compounds, will alleviate symptoms of fluoride toxicosis [illegible] confined experimental conditions, the practice has not been thoroughly tested under field conditions. More studies of the amounts and effects of the total fluoride intake from all combined sources under field conditions are needed. Likewise, the biological availability of various types of fluorides from different sources needs to be further ascertained.

More effort needs to be directed toward determining the mechanism and pathogenesis of fluoride effects. The mode of action of fluoroorganic pesticides needs additional study. Is fluorine itself the active agent or is some other substance in the pesticide involved?

Additional information on the effects of intermittent ingestion of fluorides, particularly seasonal variations in intake of high-fluoride content feed and water, is needed. Geothermal energy developments may release waters having high fluoride contents that may be consumed by animals or that aquatic species may be exposed to. The relation of this potentially toxic water to total animal fluoride exposure should be determined.

Fluorides combine in bone to form fluoroapatite crystals that are larger and less soluble than normal. What effect does this type of bone have in animals whose productive efficiency is related to their ability to periodically mobilize large amounts of calcium from their bodies? Heavily lactating dairy cows and laying hens must utilize some of their own body stores of minerals to attain a high level of productive performance. The effect of high fluoride content in bone on such performance needs further study.

9.8.6 Humans

There are many worthwhile topics for research relating to the effects of fluorides on humans. Basic studies on some lower-life forms, if adequately planned and properly extrapolated, may give further insight into some aspects of the effects of fluorides. Accurate current data on fluoride consumption from food, water, and beverages in different geographic areas, under various economic and cultural circumstances are needed. Studies using a systematic approach based on a dose-response plan should be made on basic growth effects of fluoride on cells. The effects of fluorides on cell membrane permeability should be studied. Additional research is needed regarding long-term effects of fluoride-induced bone changes and the influence of nutrition, especially relative to fluorides' interaction with other trace elements. The role of various physiological stresses on the expression of fluoride-induced lesions needs further study. Some fluorine compounds have been used therapeutically. The mechanisms by which they supposedly aid wound healing should continually be evaluated. Additional information is needed regarding causes that may mimic the effects of fluoride on teeth. Studies relating to the effects of fluoride on biological systems should utilize levels that approximate those normally found in the environment or those that can be realistically extrapolated.

9.9 CONCLUSIONS

Fluorine is a highly reactive element that readily combines with other elements to form fluorides. Fluorides are ubiquitous in the environment and are found in substantial quantities in some minerals and rock formations. They are economically important to many industries and their usage is increasing.

Fluorides are found in all biological systems. Various factors influence biological responses of vegetation, animals, and humans to fluoride. In proper amounts they are known to be beneficial to humans, some other animals, and vegetation. In excessive amounts they are toxic to biological systems. Effects of lifelong ingestion of optimal levels of fluoride in humans include increased resistance to dental caries and, in bone, an increase in the size of the apatite crystals that are less soluble. The signs and lesions associated with excessive fluoride intake vary in magnitude and are dependent on the interactions of the factors that influence fluoride toxicosis.

The principal sources of fluoride for humans are water and food, with air usually providing only minimal amounts. Forages contaminated by some industrial effluents, dust, water, and inadequately defluorinated mineral supplements are the major fluoride sources for animals. Major sources of fluoride to vegetation include: gaseous or particulate effluents from some industrial operations, wind-blown or rain-splashed soil, and sprinkler irrigation with water of high fluoride content. Fluorides may be incorporated within the vegetation or may be only surface contaminants. Some vegetation may accumulate large amounts of fluoride without showing any visible effects. Other vegetation is more susceptible to fluorides.

Vegetation normally contains, and animals and humans normally ingest, trace amounts of fluorides with no known detrimental effects. Fluoride intake standards and comprehensive guides have been developed for use in diagnosing and evaluating biological effects of fluorides.

SECTION 9

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